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Snooping with a CRT

A nurse with access to health records by means of a computer terminal types in her boy-friend's name and finds his medical history. It is not all she would have hoped for. She breaks it off with him.

A defendant in a well publicised civil case has an alibi which the court accepts. A credit card company employee, out of curiosity, calls up his file and finds that his alibi is not borne out by the list of his transactions.

A hotel booking clerk uses a computer terminal to check on a putative guest and finds that a year ago, in a different hotel, he left without paying for his room. The guest, immediately on discovering his oversight, had paid the bill by post. But this correction never got into the computer. He is told the hotel is full.

THESE are three of the diverse ways in which computers can facilitate information-collecting in somewhat murky circumstances. The Home Office has recently published a White Paper on Computers and Privacy (Cmd 6353, 28p), and with it a summary of an interdepartmental working party's review of computer usage in the public sector, with particular reference to safeguards to protect privacy and confidentiality. Back in 1972 Sir Kenneth Younger's Committee on Privacy reported that, although there was no evidence that the private sector was using computers to threaten privacy, there was need for vigilance, in the form of an independent body to review the gathering and processing of personal information. The Younger Committee also gave the government gratuitous advice that such an independent body could, in addition, study the public sector. This White Paper advances the cause of a Data Protection Authority to oversee the handling of personal information. It also gives some reassurance that, in as far as the evidence exists, the use of computers to impinge on people's private lives is not on the increase.

Privacy is a misnomer. We are really talking of the use of personal information for purposes other than that for which it was supplied or collected, and the use of inaccurate or incomplete data where the person reported on has no chance to challenge the accuracy or completeness.

Computers is also a misnomer, so to say. Computers have not, up to the present, created new opportunities for misuse of information; they have simply made

possible quicker and larger operations, allowing instant decisions to be made. Newspapers, or gossip in the staff canteen, still have the potential for much more damaging intrusions into the life of the individual than do computers. Nonetheless the computer, with its 1984 image, attracts a lot of the fire, and is often credited with monitoring skills that it does not possess.

The average reader of *Nature* would, however, probably settle for more automation in dealing with public and private enterprise. The tedium of paying separate bills for mortgage, licences, utilities, insurance—of having different numbers for health services, tax authorities, passport, bank, telephone, credit cards—often niggles; roll on rationalisation. But one third of the population of the UK regards it as an invasion of privacy even to have publicly available lists of names and addresses such as electoral lists or telephone directories. With such extremes of opinion, is there any hope that a statutory agency such as the White Paper envisages can satisfy everyone that information is not being misused?

The Younger Committee put forward ten principles for the handling of personal information in computers, including one that "there should be arrangements whereby the subject could be told about the information held concerning him". The White Paper proposes more—"the subject should also be able to find out what has been done with the information, and to whom it has been given". In a society of technocrats, we would all receive a weekly listing of what was held in data banks about us, and by whom that information had been used; the big advantage of storing information in computers, of course, is that all usage can be logged. If we found someone had been nosing around without authority, we would call our lawyer. But that one third of the nation which does not even want names and addresses listed probably has little idea of what to do if supplied with computer print-outs. It certainly is not in the habit of consulting lawyers.

Here, then, is a real job for the Data Protection Authority—to make sure not only that everyone knows what the information they have provided is being used for, but also that ways are devised for fighting misuse at the level of the individual. Many of the biggest threats to the individual, after all, do not come from the computer at all. But perhaps an imaginatively appointed Data Protection Authority, not stuffed with "the good and the great", might lead the way towards broader initiatives to look after those in greatest danger of being trampled on.



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Can Europe clean itself up?

Despite the rhetoric that bursts forth when principles of European Economic Community action are outlined, environment policy remains something of a backward region. Progress towards common action at Community level has been tardy, not through a lack of ideas but a paucity of decisions. Paul Cheeseright reports.

IN THE EEC, environment is a policy looking for an executor. Yet opinion polls would suggest that a more vigorous approach by the Community's decision-making arm, the Council of Ministers, would not be lacking in some sort of popular acclaim. Nearly 10,000 people questioned throughout the Community in the middle of last year were asked to list the most important problems facing the Community. Second on the list of five major concerns came nature conservancy, and most of the interviewees favoured European action to deal with all of the problems.

In fact, a vast amount of work into the problems and techniques of environmental control is proceeding within the Community. The great problem, however, is that most of it is uncoordinated, and at Brussels there is simply inadequate information to gain a coherent picture. For this reason, it was agreed towards the end of last year that the European Commission, the ideas centre and civil service section of the Community, should pull together an inventory of sources of information on the environment. The data would be supplied by the nine member states and include independent research exercises. The information supplied would be processed in such a way as to be compatible with similar groundwork being undertaken for the UN international reference system on the environment.

This measure extends the interchange of information that was begun in 1973 when the Community took its first halting steps towards harmonising national legislation on the environ-

ment. When one state desires to take action in an area where the Commission has not presented proposals, the measure can only come into effect six months after the Commission has been informed and only if the Commission has presented no proposals to the Council. Under an agreement of March 1973, the Commission is in any case informed of draft laws, regulations or administrative provisions. In the first two years after the agreement it was informed by the member states of 22 laws or regulations, 67 draft provisions and six international agreements, the greater part coming from Denmark, France and Germany.

The existence of such mechanisms acts as a check on the use of environmental policy in a purely chauvinistic sense, and is an acceptance of the principle that environmental policy applied in any determined fashion is a nonsense when conceived in a purely national framework. Indeed, one of the lessons of the international conference at Stockholm in June 1972 was that in the absence of any international organisation to supervise the improvement of the European environment, only the Community can execute a policy that transcends national frontiers. Yet the Community has only limited powers, and over the next decade it seems likely that any steps taken will work towards the mitigation of problems rather than their cure or outright prevention. This is not to say that water will not become purer or the air cleaner, but it is to say that there are certain forces at work within Europe that will not be pushed aside.

Photo: BTA

Among them is the demographic trend which even before 1970 had shown that 30% of the population of France, Germany, Italy and the Benelux countries was concentrated in 9% of the available land area. Further, in the area of greatest population concentration, the birth rate was found to be more than double that of the outlying regions. The result of this trend could be to entrench the so-called Golden Triangle, the industrial area that extends from the Midlands of England to the Gulf of Genoa. Any environmental policy is inevitably linked to balanced development. But this is not taking place, and, indeed, the Community's agricultural policy is actually encouraging people off the land into the industrial conurbations.

Under the liberal capitalist economic systems of the Nine, new industries are usually attracted to the main market areas, thus strengthening the tendencies towards pollution that have been created by a century's unplanned search for industrial prosperity. In the strictest terms, then, a successful Community environment policy demands some control over the disposition of economic activity. But there is no power to create such a system of control, nor much evidence of any desire to create it. It would involve the emergence of a supranational state which, while it might be the aim of the European integrationists, is scarcely a realistic political concept for the time being. Community environment policy is likely to remain something of a patchwork while the politicians strive to come to terms with what the Community's Altiero Spinelli once called "a spectrum of complex interdisciplinary problems, whose impact, interconnection and consequences are frequently not completely known as yet." There is the additional problem that the environment as such is not mentioned in the Treaty of Rome and that, freed from the legal requirements of common action, the member states' movements on an integrated policy will be a direct reflection of their somewhat haphazard desire to move closer together politically.

It is against this background that the Community has been formulating and enacting a limited environment policy. The Community was a relative late-comer to the field. It was not until 1971 that the Commission established a special unit to deal with policy, and it was only at the Community summit meeting of October 1972 that their efforts received much political recognition. A detailed action programme was ordered for presentation in the middle of 1973. This programme was adopted later and ran until the end of 1975. Now a further programme to cover the next five years, with revision after

2½ years, is under consideration.

The main thrust of the Community's first programme covered three broad areas. In the first place the aim was to prevent or reduce pollution; the second aim was to improve the environment generally and then, finally, to push forward Community involvement in the framework of international action on the environment. Within these general areas, the Community adopted the principle that "the polluter pays". This may turn out to be something of a misnomer, for the real fact is that the consumer pays. Where factories or agencies take steps to minimise their pollution of the environment, it is inevitable that this will be reflected in the prices charged for the products created. In addition, governments are using taxpayers' money to aid companies introducing anti-pollutant measures. The Community has since the end of 1974 had a general rule as to the apportionment of costs and the role of the public sector in providing aids for anti-pollutant measures. But this measure is less concerned with safeguarding public money than preventing the companies of one country gaining a competitive advantage over those of another.

The Community has also adopted a set of rather obvious principles for the administration of environment policy. There are eight in all, aimed above all at the co-ordination of national programmes. The use of natural resources which damage the ecological balance should be avoided, environmental damage should be prevented rather than corrected afterwards, technical progress must include efforts to protect the environment, the polluter should pay, the environment will be taken into consideration at an early stage of technical planning, activities in one country should not damage another and there should be nothing to impede the operation of the common market. The principles are banal, but significant in the European context mainly because they had not been enunciated before.

At the Community level, numerous research programmes have been set moving and plans for a European Foundation for the Improvement of Living and Working Conditions have been laid so that the Foundation should start working early this year. The Council has also adopted a resolution on the desirable quality of surface waters required for drinking and a recommendation on the storage and treatment of waste oil, and has adopted the Paris Convention for the prevention of marine pollution from land-based sources. At the end of 1975, the Council agreed that the Commission should represent the Community in international negotiations aimed at providing a basis on which it will be

possible to keep the Mediterranean cleared of pollution emanating from the land. In this case, the Commission's mandate is an extension of the agreement that was reached at the Paris Convention, which covered the North-East Atlantic. These examples give a flavour of the breadth of the work that is being undertaken at the Community level, although it can be argued cogently that the effort is minimal when related to the scale of the problems to be solved and the power of the Community when acting in a united fashion to solve them.

6 Given a problem it is possible to suggest a scientific counteraction. But Community problems are not dealt with according to such simple terms 9

But this really points up the difficulties that the Community is facing. Given a problem it is possible to suggest a scientific counteraction. But Community problems are not dealt with according to such simple terms. In the first place, there might be, and often is, argument about the scientific response to the problems because the geographical nature of the Community is so varied. In the second place, any scientific response to a problem has to be consistent with the overriding economic aims of the Community. Of course, the Community is concerned with economic growth, but the difficulty goes deeper than that into the existence of the common market. It is a dominant principle that the common market should not be endangered, that there should be free traffic in goods and that no company or country should have an advantage over another, created by the Community rules.

Two incidents in recent months provide evidence of the dilemma created by these circumstances in the Community. One instance remains unsolved. The other has been widened to embrace a compromise that might embrace the environmental end, while taking into account the nationally-based scientific and economic means.

The first relates to the content of lead in petrol, a subject which the Community has broached but has never been able to follow through because of the opposing national attitudes and interests. At the beginning of the year the West German Government was due to introduce a new regime governing the extent of lead permitted. In introducing stricter measures in the interests of curbing air pollution, the Germans have pursued a policy that is further advanced than that of other Community states. On January 1, the Germans reduced the legal lead content in petrol to 0.15 grams per litre from

0.4 grams. Precisely because the German regulations will be stepping outside the commercially accepted norms, the Commission has received complaints from a group of German oil companies and a number of Dutch firms that, as they are unable to meet the 0.15 grams regulations, they are being forced out of the market. Therefore, the companies argue, the regulation is an infringement of the principles of the common market and free competition. The case remains unresolved.

The second instance concerns the emission of toxic waste into the aquatic environment. It was the subject of bitter dispute between Britain and the other eight members of the Community at a Council meeting in October. A compromise was reached in December. Broadly the eight members wanted discharge controls for pollutants like cadmium, mercury, organohalogenic, organophosphoric and organotitanic composites, and a list of others making up a so-called black list. Britain, on the other hand, argued that there should be an observance of quality control objectives—in other words, there should be no control on discharge where it could be indicated that the discharge did not affect the immediate environment. The eight contended that unless there was a uniform emission standard there would be distortion of economic competition. The British rejoinder was that this was nonsense and that there should be recognition that geographic factors were different in the varied parts of the Community. Britain had short rivers, a long shoreline and not many factories on the rivers. The compromise finally agreed was that emission standards should be used but there could be exceptions where quality objectives may be used.

While the Community, it may be argued, lost a chance, because of British intransigence, to move forward into the area of positive controls, it came face to face with the fact that environmental policy will have to be flexible in the future. The conditions in the north of Scotland are not the same as those in Sicily. If fish are not given a chance to live in the Rhine, they are breeding more heavily in the Thames. But beyond this, the flexibility of the approach to aquatic pollution is probably more in keeping with the political realities of 1975-6.

In a general sense, the environmental lobby is not as strong as it was in 1972-3, except insofar as it can bring its power to bear on local issues of immediate importance. And even if it was, it would not necessarily make much difference. Environmental policy in the Community has less to do with science and more to do with politics and economics. □

international news

Just four years ago, President Nixon signed into law a piece of legislation which has fundamentally changed government support of biomedical research in the United States. Called the National Cancer Act, the legislation was designed, in Mr Nixon's words, to launch "a great crusade against cancer". The crusade has always been controversial, causing deep divisions within the biomedical research community about how it should be managed. But it has recently come in for some fresh criticism which bears especially close watching. In fact, the indications are that a major political debate about the structure and scope of the cancer research programme is beginning to take shape.

Even before Mr Nixon put his signature to the National Cancer Act, the measure was controversial. The chief bone of contention in the early days was the justifiable resistance within the scientific community to the notion that cancer research could be managed like an Apollo-style effort to land men on the Moon. Though concerns over the NASA-like approach persist, frequently finding expression in attacks on the huge amount of money being spent on efforts to track down human cancer viruses, criticisms of the cancer research crusade have recently coalesced around two arguments, both of which have been raised in debates in Congress in the past few weeks. The first is the argument that rapid increases in funds for cancer research have siphoned money from other, equally deserving, research programmes. And the second is the contention that the cancer crusade has concentrated on an elusive search for cures and treatments at the expense of prevention—in short, environmental causes of cancer have been relatively neglected.

The first complaint is by no means novel. In fact it was raised three years ago when Mr Nixon cut back research budgets across the board but spared the politically sensitive cancer programme from the axe. The matter took on a new dimension last September, however, when a group of Senators, led by Gaylord Nelson of Wisconsin and Alan Cranston of California, proposed an amendment to a budget bill which would have pared down the budget for the National Cancer Institute and redistributed some of the savings to other institutes of the National Institutes of Health (NIH).

The amendment was soundly defeated, but the issue of research priorities got a good airing on the Senate floor.

Armed with a sheaf of statistics and with letters of support from former NIH directors and top government health officials, Nelson and Cranston provided details of how the budgets have grown for cancer research and, to a lesser extent heart research (which was aided by the National Heart, Lung and Blood Act of 1972), while budgets

Cancer controversy recommences

by Colin Norman, Washington

for other NIH institutes have languished. Between 1970 and 1975, the budget for the National Cancer Institute (NCI) rose by 280%, from \$182 million to \$669 million, and the budget for the National Heart and Lung Institute (NHLI) increased from \$159 million to \$303 million, while spending on all the other 11 institutes rose by only about 20%, from \$750 to \$908 million. In constant dollar terms, NCI's budget rose by 186%, NHLI's increased by 52%, while the rest of NIH suffered a 13% drop in funds.

Although few Senators were willing to vote for a redistribution of funds to less politically favoured research programmes, the matter of biomedical research priorities is now under intense scrutiny by a top-level Presidential commission which is examining NIH's research policies. Established last year by a bill sponsored by Senator Edward Kennedy, the commission is due to issue a report by April 30, and its findings are likely to spark a fundamental re-examination of biomedical research policies and priorities.

The second major complaint raised recently about the cancer crusade is that the effort is devoting insufficient attention to finding ways to prevent, rather than to treat or cure, cancers. The impetus for such complaints is generated by two factors. The first is the growing realisation that the majority of human cancers may be linked to environmental factors, such as carcinogens encountered in the work place and in the general environment (including cigarette smoke). The second is the fact that in spite of massive expenditures on cancer research, survival rates for people suffering from

many of the leading cancer killers have improved little since the 1950s.

Thus, Russell Train, the head of the Environmental Protection Agency, pointed out in a recent speech that as many as 60–90% of human cancers can be traced to environmental causes, and he suggested that "we may be approaching the whole question of human health from the wrong side . . . an ounce of prevention may well be worth a pound of cure".

Similar sentiments were expressed more bluntly last year by a sub-committee of the National Cancer Advisory Board, NCI's top advisory body. The sub-committee, which was chaired by Dr Philippe Shubik of the University of Nebraska, expressed its "sense of general astonishment" that the cancer programme "does not appear to have accorded an adequate priority nor sense of urgency to the field of environmental carcinogenesis". The sub-committee's report went on to recommend that more money be put into chemical carcinogenesis (it estimated that less than 10% of NCI's budget is now spent on such studies) and it suggested that if necessary some money should be reprogrammed from other fields, such as tumour virology.

NCI officials have generally countered that they are spending much more than 10% of their budget on environmental carcinogenesis, and that if good research proposals in that field come along, they will certainly be funded. Nevertheless, complaints about lack of attention to environmental carcinogenesis have percolated through to Capitol Hill, and last month Congress approved an appropriations bill for NIH (which has since been vetoed by President Ford), which contained \$3 million to establish a research programme on job-related cancers.

With discoveries of new occupational and environmental carcinogens—such as the link between vinyl chloride gas and liver cancer in plastics workers—continuing to flood in, the death rate from cancer continuing to increase (it rose dramatically in the first six months of 1975 in the US, but the increase may be a statistical quirk), and the cancer programme continuing to occupy a politically favoured position in the federal budget, it seems inevitable that there will be a renewed political debate about the structure and scope of the cancer programme this year. □

DELEGATES from the contracting parties to the London Dumping Convention 1972, along with observers from other countries and interested international organisations, met for the first time last month in London to choose an appropriate organisation to undertake its secretariat duties and to begin preparations for its first substantive meeting later this year.

The world-wide Convention—its full title is the Convention on the Prevention of Marine Pollution by Dumping of Wastes and Other Matter, London 1972—has been signed by 54 states, 22 of which have ratified it. The Convention prohibits the dumping at sea of certain dangerous substances, including mercury and cadmium and their compounds, organohalogen compounds and highly radioactive materials, and subjects the dumping of a large number of other substances to stringent control by the issue of specific permits by national authorities.

The conference agreed to a joint proposal from Britain and Mexico that the secretariat's work should be given to the Inter-Governmental Maritime Consultative Organisation (IMCO), the London-based UN agency with over 90 members which has until now administered conventions regarding marine pollution from ships and operated in the fields of marine safety and navigation.

The conference set up a working committee to examine the plan, and also agreed that the first substantive meeting of contracting parties to the Convention should be held in September.

- The enforcement from the beginning of January of further provisions of the Control of Pollution Act 1974, which covers waste disposal and reclamation, noise, water and air pollution, has coincided with the Department of the Environment's latest survey revealing a further slight fall in the extent to which the UK's rivers and canals are polluted.

A total of 840 miles of river and canal is now classed as "grossly polluted", a reduction of 46 miles. The improvement has increased marginally the lengths of river classed as "poor quality requiring urgent improvement", but at the other extreme an extra 160 miles have been added to the lengths considered free from pollution.

The survey is based on tests carried out in 1973. The improvement on the previous survey in 1972—the first was made in 1970—was less marked in tidal rivers and canals than in non-tidal rivers. And altogether a total of 182 miles was downgraded.

- The reports of three separate committees examining lead levels in food were published last month when the Working Party on the Monitoring of Foodstuffs for Heavy Metals carried in its fifth report (*Survey of Lead in Food: First Supplementary Report*) two appendices containing the advice of the Food Additives and Contaminants Committee and of the

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toxicity sub-committee of the Committee on Medical Aspects of Chemicals in Food and the Environment.

The Working Party, whose second report, in 1972, was also a survey of lead in food, concluded that the amounts of lead in the average diet in the UK had not increased since then, and that the mean lead concentration in canned infant foods had significantly decreased. The toxicity sub-committee, which advises the Food Additives and Contaminants Committee and various government departments on the toxicological aspects of the use or presence of additives and pollutants in food and the environment, considered that the present intake of lead from food in the UK was unlikely to constitute a hazard to the health of the general population.

The Working Party's surveys, involving the analysis of some 6,000 samples of foodstuffs and nearly 12,000 samples of drinking water, showed that the average consumer's lead intake was less than half the 'provisional tolerable weekly intake' of 3 mg for adults, published by the FAO/WHO.

But the Food Additives and Contaminants Committee, although endorsing the underlying view, recommended reductions in the maximum permitted amounts of lead in food in its separately published *Review of the Lead in Food Regulations*. Margins of safety were not as great as could be desired, the

committee argued. It wanted ministers to enforce a limit of one part per million for most foods, a lower limit for baby foods, an end to the exemption from all limits of fish in which lead occurs naturally, and extra efforts to cut the amounts of lead in tinned foods.

- Just a couple of weeks after the announcement that Lord Beswick would head the new publicly owned corporation British Aerospace when it is formed—an appointment that attracted some Opposition criticism because he has not held an executive position of any sort in industry—the Labour Government named the board members of the new British National Oil Corporation (BNOC) whose first task will be to organise the structure of the state oil undertaking. And on the list so far is not a single senior oil industry management executive.

Indeed, the only man with any remotely practical experience in the area seems to be Mr Denis Rooke, chairman-designate of the British Gas Corporation. But his appointment, like that of the chief executive of the Shetland Island Council, drew speculation about conflicts of interest as well as about BNOC's possible influence over British Gas. And the inclusion of Lord Balogh as deputy chairman, a part-time post, has raised questions about BNOC's independence from government interference, because although he has retired as Minister of State at the Department of Energy (headed by Mr Anthony Wedgwood Benn), Lord Balogh remains Mr Benn's special adviser.

The chairman of the board, which also includes a lawyer, a trade union leader and several civil servants, is Lord Kearton, formerly head of Courtaulds.

- Dr Walter Marshall, the Director of Harwell, the Atomic Energy Research Establishment, is relinquishing his position (though not, apparently, his office) there to become deputy chairman of the UK Atomic Energy Authority (UKAEA) on Mr Frank Doggart's retirement. He will retain his other major post, that of chief scientific adviser to the Department of Energy, except when it comes to nuclear matters, which are the province of the UKAEA chairman, Sir John Hill.

The move, announced by Mr Benn, marks a large promotion. Dr Marshall was, at 43, the most junior member of the UKAEA Board; he now has responsibility for scientific and technical policy throughout the organisation. Dr Lewis Roberts will take his place as director of Harwell.

AS THE Council for Mutual Assistance (Comecon) countries considered the end of their 1971-75 Five-year Plans and formulated their plans for the coming five years, a whole series of meetings, conferences and discussions were held of the Standing Comecon Commissions. Plans have been put forward on such diverse matters as the unification of standards for industrial production, the approval of new drugs to be used in the treatment of malignant tumours, the division of labour between the participating countries in the manufacture of electronic components, and specialisation and co-operation in the manufacture of instruments for the artificial insemination of cattle.

For the first time, the new plans will include the construction of joint industrial projects by a number of member countries. And the non-European members of Comecon are not forgotten. Within the framework of the existing scheme for the integration of information systems, Czechoslovakia and Hungary are to provide information systems for Cuba, and the Soviet Union will provide Cuba and Mongolia with industrial, agricultural, and transport equipment in return for basic exports and raw materials.

● The new Polish five-year plan, as laid down by the Seventh Congress of the Polish United Workers' Party which met in Warsaw from December 8-12, envisages a considerable expansion of investment in science. This is despite the fact that the growth of the Polish economy in the five years of the Gierk regime has been facilitated by foreign borrowings which, in bringing

demands for interest payments, will make higher prices and food shortages likely over the next five years.

Comecon diary

from Vera Rich, London



Expenditure on both science and technology is put at 200,000 million zloty, after 115,000 million in the previous five years. Special emphasis is to be placed on research into the more efficient use of fuels, raw materials and water resources, improving the technology of the coal and copper industries, the introduction of aluminium production from native ores and the production of new materials for the electronics industry. The application of science to agriculture, public and occupational health and child care are also mentioned.

According to Sylwester Kaliski, the Minister of Science, Higher Education and Technology, the plans will be im-

plemented through a new scheme which, with cooperation between scientists and administrators, will lay the foundation of a "strategy for scientific research and technical advance, a plan composed of government programmes, departmental problems and basic research". This, he said, would unify the whole activity of research and its practical implementations in Poland.

The Polish Academy of Sciences is to play an important role in the research part of this programme, and it is introduced also to involve the universities.

● In the German Democratic Republic's economic plan for 1976, which (if successful) will result in a 5.3% increase in the national income, a 6% increase in the production of industrial goods and an improvement in labour productivity of 5.5%, the plan for science and technology envisages more than 160 specific tasks to be fulfilled on time and to a high standard. The "tasks" cover a wide range of applied science, from improved fuel economy in power stations to rationalisation of the washing-powder industry.

The attainment, or otherwise, of the proposed targets may itself be somewhat difficult to evaluate, however. According to the East Berlin Central Statistics Board, a number of enterprises in the GDR have been "interpreting in their own special way" their economic results for 1975, so that in many cases the figures submitted make the drawing of any sound economic conclusions problematic. The board urges exact calculation, rather than "interpretation", of the figures.

Indian states attack population growth

from Our Correspondent, Jullundur

AFTER more than two decades and the expenditure of thousands of millions of rupees, India's family planning programme has achieved only limited success. But Draconian measures proposed by two state governments may well change all that through a revamped and much more severe stick-and-carrot policy to encourage sterilisation.

Taken up officially in 1952, the Indian birth control programme started off modestly, but only received its first big push after the publication of the 1961 census data, which showed a higher-than-expected rate of population growth. The programme has nevertheless made only slow progress. According to official figures, the present birth and death rates are 36.6 and 16.9 per thousand respectively. Due to improvement in health services, the

death rate has fallen and is expected to go down even further. But the slow decline of the birth rate can be attributed to a number of factors. Paucity of funds, inadequate communications and lack of official enthusiasm at lower levels have proved obstructive; but poverty, malnutrition, ignorance, superstition and illiteracy are the more formidable hurdles. There have been suggestions that new families be allowed to have no more than two children. But so far the government has steered clear of introducing compulsion into the programme, even though the population increases each year by an amount equivalent to the population of the whole of Australia.

The state governments of Haryana and Punjab, however, have decided on steps which could spell the beginning of a new trend. The Haryana government has announced a series of measures to promote smaller, two-children families by means of incentives and disincentives. The state has set itself a target of reducing its net

rate of population growth to zero before 1985.

The incentives include cash prizes worth 100,000, 75,000 and 50,000 rupees, which will be awarded each year to three districts in the state which capture top spots in family planning performance over and above their allotted targets. Factories in the state, where all 'eligible' workers accept sterilisations after two children, will qualify for benefits from a 'Labour Welfare Fund', and the best village-level governing bodies in each district will also be eligible for cash bonuses. All children of 'eligible' government employees will be entitled to reimbursements of medical expenses up to the age of 5; thereafter only two of them will be given this facility. Employees who already have more than two children will only receive medical reimbursements if they are sterilised within a year of the date of enforcement of these decisions.

The disincentives are aimed primarily at government employees with

more than two children who fall into the category of 'eligible' couples. Unless they get themselves sterilised within 12 months, they will not be allotted government accommodation, or, if they are already in possession of government accommodation, they will be charged standard rent; they will not be allowed any house-building advance, or loans for buying wheat or vehicles; and on being transferred, they will receive travelling allowances only for two of their children. New recruits into Haryana government service will have to undertake not to produce more than two children. A third child will render them liable to dismissal.

Days before the Haryana government decisions, the Punjab government had announced similar measures to curb the rate of population growth in that state. Among other things, women employees in Punjab would no longer be provided the facility of maternity leave and other related benefits beyond the second child. □

Commission foresees 200-mile limit

THE European Commission has recently disclosed details of proposals regarding European fishing policy, grasping in the process the nettle that the problem of 200-mile fishing limits represents.

The plans, which now require a decision from the nine member governments, envisage a time when 200-mile fishing limits are approved—most likely at a forthcoming session of the UN Law of the Sea Conference. Member governments are enjoined to formulate a common position on the issue.

The Commission emphasises the need for conservation of fish resources in its proposals, and this would probably involve setting catch quotas. The inland waters of each of the nine member states should, it says, be opened to fishermen from other member states

(this is due to happen after 1982), and argues that special arrangements will have to be made to protect inshore fishermen and preserve fish stocks when limits are extended to 200 miles.

Quotas would have to be negotiated with non-member countries like Spain and Norway, the Commission says, and access terms for Community fishermen in their waters would also have to be agreed.

In Norway itself, meanwhile, the minister in charge of questions relating to the law of the sea, Mr Jens Evensen, has spoken about the sort of agreements he foresees regarding conservation and quotas. He was indicating new guidelines for Norway's negotiations with 15 countries about the establishment of its proposed 200-mile economic zone. The total catch, he said, should be allotted to the various countries with a clear preference for the coastal states. And he described the EEC fisheries policies as a "heavy burden for Europe". □

OPEN season on the Food and Drug Administration (FDA) is, as usual, in full swing; cannon to right of them, cannon to left of them, volley and thunder, in the "health food" magazines, in *Science*, where the anti-everything editorial staff have long used FDA as a favourite target, and even in *Nature*.

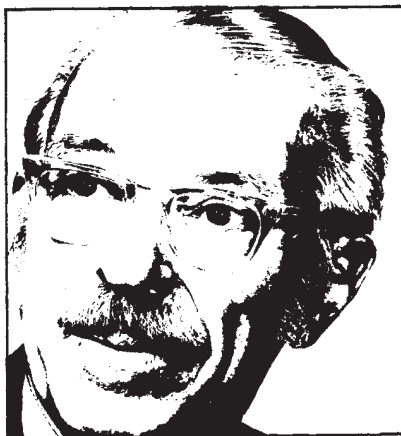
A new report on cyclamates says that "a blue-ribbon scientific panel" tentatively concluded on December 10 (with one dissident) that more than 20 new studies with animals failed to show cancer, birth defects or genetic damage. Now what happens? If cyclamates are put back in soft drinks, the Center for Lawyers in the Performing Arts will erupt in rage, aided by FDA's in-house consumerist, who will inject chick embryos with cyclamates. The embryos will die. Chickens, unlike dogs, are expendable. (But what dog ever laid a nice, fresh breakfast egg?) The FDA will be accused of collaborating and conniving with the pharmaceutical industry to put a poison in our food.

However, if cyclamates are not put back in soft drinks, the Toby Belch Society, or some other watchful guardian of the obese, will protest angrily. The FDA will be accused of plotting and conspiring with the sugar industry to keep a harmless sweetener from people who need it in reducing diets. The FDA must indeed feel grateful for the existence of cyclohexylamine, a metabolic product of cyclamates that may give the regulators a little breathing spell while it is further investigated. Few of the critics will bother to read the Federal

Food, Drug and Cosmetic Act, which gives very specific directives for evaluating applications for the use of food additives.

Some confusion in this general field undoubtedly arises from lack of understanding of the "GRAS"

The policeman's lot



THOMAS H. JUKES

(generally recognised as safe) list. This list, which is currently being overhauled, includes about 670 substances recognised in 1958 as suitable for food, compiled from FDA food standards, from State regulations, and from lists of substances "known to have been used in food for some years without reported adverse effects." Long custom rather than scientific investigation led to the acceptance of most of these as (or in) foods. Undoubtedly, many of them would not "make it" if intro-

duced as "new" food additives. The FDA appears to be getting blamed for allowing bacon, containing mutagenic substances, to be sold. Just who got the idea of soaking meat in nitrates to preserve it is not clear, but the practice certainly antedates the FDA, and the present century. For that matter, the treatment of meat with smoke adds carcinogens to the diet, and this process must have originated in the caves of our remote ancestors.

The FDA points out that, for all food additives, new information casting doubt on safety will lead to "necessary measures provided for in the law to be undertaken to protect the public health." Actually, some additives, such as safrole, have been quietly dropped. There are no provisions, of course, for using new information of a positive nature, such as the increase in mean life span of mice, and the partial suppression of carcinogenicity of certain aromatic amines, both reported for the antioxidant butylated hydroxytoluene (BHT), whose name is enough to make it suspect, regardless of its properties.

Meanwhile, FDA is still ducking bullets on other issues, as described in *Nature* (November 20). Charges of "industry favouritism" by disgruntled employees make great headlines, and nothing is duller than a rebuttal. Industry, of course, says it is strait-jacketed by FDA. The pushers of the quack cancer remedy "Laetrile" say they are, too, as they lie a-basking in the sun, south of the border, down Mexico way. The policeman's lot is not a happy one.

correspondence

Higher education

SIR,—May I comment on two related articles on higher education which have appeared in recent issues of *Nature* (November 13 and 20)? The question of whether universities should be concerned with "education" or with professional "training" is raised in relation to the balance of faculties and the cost to the taxpayer.

In my view there is a need for two distinct types of higher educational institution. One of these, traditionally universities, should be principally concerned with scholarship and furthering the bounds of human knowledge, and with passing on the fruits of such knowledge and research, through teaching, to any who are sufficiently interested and wish to receive it. Examinations, if they are appropriate here at all, should not take the form of professional qualifications, but should rather be used for selecting academics to continue the work of the university.

The other type of institution should have the specific function of training for professional qualifications. Such an institution might be a polytechnic where the teaching should be geared to qualifying examinations for professional men expecting to practise in medicine, the law, sciences, engineering, business management or whatever. The teachers should have had some years of practical experience and they should constantly refresh that experience on a sabbatical principle or by periodical exchanges with practitioners. Research work should be aimed at practical application in response to the needs of industry, hospitals and so on.

Universities should be reduced in number and much reduced in size, but the balance of faculties should certainly be maintained. This would provide an environment for the broad education of relatively few who are academically inclined and who would be expected to achieve the highest standards of excellence in scholarship, imagination and innovation.

Polytechnics could afford to be more specialised and might benefit from it. In order that the trainees should not suffer from a lack of education in the broader sense, the polytechnics should allocate some course time to a variety of subjects for which the universities could send lecturers and set up

seminars. There would be no examinations in these extra-curricular subjects and a wide choice should be offered.

The courses offered by polytechnics should ensure that a sound basis of theory and practice is achieved, and the chartered professional bodies should have a considerable voice in the curricula and in the form and content of the qualifying examinations. In some professions where developments are rapid, it might be desirable to insist on further training and examination at intervals to ensure that high professional standards are maintained throughout a career.

Developments in the future along these lines might ensure better academic research, better professional standards, and give better value for the taxpayer's contribution to higher education.

Yours faithfully,

A. C. MASON

Wirral, Merseyside, UK

The protein gap

SIR,—Man has always desired simple answers to the complex problems of the world; to reduce everything to a common denominator. Many have done this with regard to nutritional problems, claiming over the past twenty years that protein was the only answer. As Drs Waterlow and Payne point out in their article "The Protein Gap" (November 13), this is not true. Unfortunately they then fall into the same error by concluding "the protein gap is a myth and what really exists . . . is a food gap and an energy gap". The new simple answer for them is energy.

Protein-energy malnutrition, be it kwashiorkor or marasmus, has never been, except in the rarest of cases, a single nutrient deficiency disease. It has always involved the lack of vitamins and minerals as well as energy and/or protein and all the other factors required for good nutrition.

To talk only of energy and protein as the FAO/WHO report did, and as the authors do, is meaningless. To suggest merely that more of traditional diets is all that is needed is, in most cases, no solution to nutritional problems. Many of these diets were shown to be inadequate by early workers such as McCarrison and they continue to be shown so in current studies.

Nutrition deficiencies are encoun-

tered primarily in populations with a limited choice of foods. Adequacy is best assured through the use of a wide variety of foods having complementary patterns of nutrients, as has been pointed out by the US National Academy of Sciences. This means improving traditional dietary patterns.

Until man is willing to reckon with the complexity of the nutrition problem and to deal with it as such, he is doomed to repeat the errors of the past.

Yours faithfully,

WALTER J. BRAY

Department of Food Science,
University of Reading, UK

Human consumption

SIR,—The statistic of the Potato Marketing Board quoted in *Nature* (December 11, page 484), and quoted here in full,

"Contrary to the trends in all other countries in the European Economic Community the rate of movement into human consumption in Great Britain increased during the three previous seasons (and shows a provisional offtake for 1974/75 of 222 lb per head per annum)", may suggest spud-deficiency to you, but it may imply an alarming increase of cannibalism to others.

Yours faithfully,

JOEL J. LLOYD

National Academy of Sciences,
Washington, DC 20418

Peace Prize

SIR,—Your leader (October 16) concerning the awarding of the Nobel Peace Prize must be the ultimate expression of English diplomacy. Tell me, what better example anywhere of peaceful struggle for freedom is there than Sakharov's? No one would have to lose any freedom themselves to satisfy his requests, and unlike some prizewinners he would not have to lay down his gun to pick up the prize. Yet you say it shouldn't be given because it might be offensive to the bureaucracy of the Soviet Union. What utter hypocrisy for such an editorial to appear at such a time.

Yours faithfully,

FRANK SORESENSEN

Corvallis, Oregon,
USA

news and views

High resolution interferometry

from F. G. Smith

THE angular accuracy of radio astronomical maps of celestial objects has progressed beyond all optical measurement, but it has reached a natural limit. This limit is provided by the available intercontinental distances between the component radiotelescopes used in Very Long Baseline Interferometry (VLBI). The observations reported on page 17 use multiple baselines between the 100-m radiotelescope in Germany and three American radiotelescopes, at Green Bank, Owens Valley, and Fort Davis. At a wavelength of 2.8 cm a source diameter of only 0.25×10^{-3} " can be resolved by this multi-element interferometer, which uses all of the six independent baselines between the four telescopes. The combined efforts of 13 radioastronomers, and doubtless many supporting staff, at these telescopes now give us a map of the central radio source in the galaxy NGC1275, well known optically as a Seyfert galaxy with very large internal velocities. The radio map only covers the nucleus of the galaxy, with a total extent of 5 pc, but the resolution is 0.1 pc. Longer

wavelength radio emission, as mapped by the 5-km radiotelescope at Cambridge, comes from much more extensive regions, while the whole galaxy extends to a diameter of more than 50 kpc. It is the very core of the galaxy that is being explored in this new map.

The technique of VLBI does not give the same full and unambiguous map as does aperture synthesis. For example, the nucleus of NGC1275 is elongated, with components whose sizes, shapes and relative dispositions are now fairly well determined: but it would be equally possible for the whole map to be rotated by 180° . This is of no consequence in the physics of this radio source, which is known to be active on a very short time scale. Most of the radio emission has appeared during the last 15 yr, since the time that measurements started on short wavelengths.

Rapidly changing radio sources present further problems in interpreting VLBI measurements. If a single baseline is used for measuring an apparent

source diameter, the source can appear to grow or shrink alarmingly rapidly.

An effect of this sort has even led to the suggestion that some components of radio galaxies are expanding outwards with velocities greater than that of light. Such an interpretation requires the various components of a source to retain their relative intensities, and to change only their relative positions. The isolation of several small components within the nucleus of NGC1275, which must be the seat of the large intensity changes observed since 1960, suggests that it is premature to interpret any changes of configuration of radio galaxies as due to "super-light" velocities.

The physical processes in the nucleus will provide much food for thought. Large parts of the galaxy are moving with relative velocities of over $3,000 \text{ km s}^{-1}$. Many theorists consider that the kinetic energy of this motion comes from an explosive event in the nucleus, where the radio emission now demonstrates the active generation of high energy particles and magnetic fields.

Behaviour of the ionosphere and plasmasphere during geomagnetic storms

from T. B. Jones

THE interaction of the solar wind with the Earth's outer environment involves complicated processes many of which are not yet fully understood. The geomagnetic and ionospheric storms produced by enhancements of the solar wind are particularly interesting since they give rise to major changes in both the ionosphere and magnetosphere. These in turn, disrupt long range radio communications and navigation systems which depend on the reflection of radio waves from the ionosphere.

Solar disturbances frequently produce an increase in the velocity and concentration of the particles of the solar wind. When this enhanced wind

reaches the Earth's magnetosphere it produces several phenomena which include modification of the Earth's field, increased auroral activity and marked changes in the electron density distribution of the ionosphere. The arrival of the enhanced solar wind at the magnetosphere boundary increases the compression of the geomagnetic field which is observed as a sudden commencement geomagnetic storm on the Earth's surface. Simultaneously, the number of energetic particles in the magnetosphere trapping regions increases as particles are injected into the magnetosphere by transport across the magnetic shells. The increased ring current due to the particle enhance-

ment is responsible for the main phase of the storm. A detailed account of the dynamical processes which govern the behaviour of the trapped particle belts can be found in *Particle Diffusion in the Radiation Belts*, (Schulz, Lanzerotti and Roederer, Springer-Verlag, 1974).

Changes of shorter duration known as 'polar substorms' can occur in the polar magnetic field. These disturbances are a feature of the 'auroral oval' which corresponds to the region where the field lines that leave the Earth's surface stretch out into the magnetotail. The role of the north-south component of the interplanetary magnetic field in substorm processes

has been the object of some controversy. It is generally thought that the southward turning of the field leads directly to a series of growth processes which trigger the substorm. On the other hand measurements in Alaska indicate that substorms occur frequently even when the field component is directed northwards. This conflict has been resolved by Akasofu (*Nature*, **286**, 191; 1975) who suggests that the occurrence of a substorm depends neither on the field direction nor on its rate of change, except that if a steady northward field (>6 h) is established then no substorms are observed. During these events energetic particles travelling along the magnetic field lines penetrate into the lower ionosphere where they produce ionisation enhancements in both D and E regions which greatly affect the propagation of radio waves through these regions. It has been suggested that even lower levels of the atmosphere are influenced by geomagnetic storms. Olson (*Nature*, **257**, 113; 1975) has found that at tropospheric heights, the 500 mbar vorticity index, which is a rough measure of cyclonic activity in the Northern hemisphere, decreases during the main phase of a storm.

Storm-induced disturbances are not confined to polar latitudes and the F region of the ionosphere is affected even at the magnetic equator. Both increases and decreases in this layer's electron density are observed depending on the particular storm event. The detailed mechanisms of F region storms have been discussed by Rishbeth (*J. atmos. terr. Phys.*, **37**, 1055; 1975) and changes in thermospheric circulation are shown to be a major factor in producing the observed ionospheric disturbances. In principle, both positive and negative storms can be caused by circulation changes though it is likely that other factors such as electromagnetic drift, ionosphere-magnetosphere plasma flux and the effects of propagating gravity waves have important roles especially at low latitudes. Storm-induced exchanges of ionisation between the ionosphere and protosphere have been observed by Soicher in a recent satellite experiment (page 33, this issue).

In view of the complexity of the storm processes there is an urgent need for further measurements of particle fluxes, magnetic field variations and ionisation changes. An international cooperative effort to obtain these observations will be made during the forthcoming International Magnetosphere Study (IMS), period 1976 to 1978. A wide range of ground based and space vehicle techniques will be deployed in a coordinated programme to study the magnetosphere and its

influences on other regions of the Earth's environment. □

Weak gamma-ray bursts

from A. C. Fabian

SOURCE counts are widely used by astronomers working in all parts of the spectrum, and appear under various names and guises. Comparisons of the number of faint sources with brighter ones can yield information on the geometrical distribution of sources in space, their evolution with time, cosmological effects and the distribution of absorbing material. This information is gained without any precise knowledge of the distances to the sources. Unfortunately, however, source counts do not give unique solutions to problems. This is mostly due to the fact that there is always some spread in the intrinsic luminosities of the sources.

The latest category of events to be counted are cosmic gamma-ray bursts. These are the enigmatic flashes of gamma radiation first detected by the Vela satellites. The only facts really known about them are that they show complex structure on time scales of hundredths of seconds to tens of seconds, their spectra somewhat resemble those expected from thermal bremsstrahlung from regions of temperature $\sim 10^8$ K, and their origins lie outside the inner Solar System. Counts of the more intense bursts detected by the Vela and other satellites, with integrated fluxes greater than $\sim 10^{-4}$ erg cm^{-2} , suggest that they are relatively uniformly distributed in space. The rate is such that weaker events ($\sim 10^{-7}$ erg cm^{-2}) are predicted to occur several times per hour. Such a prediction is testable by balloon-borne detectors, and Bewick, Coe, Mills and Quenby of Imperial College London have recently reported the results of a series of flights (*Nature*, **258**, 686; 1975).

Only one event was observed, of size 1.2×10^{-7} erg cm^{-2} , in 13.5 h of observing time. This is a significantly different rate to that predicted. In the authors' opinion it suggests that a uniform source distribution can be excluded. Such a result might be expected if the sources are galactic, for then there might be fewer weaker sources observed in directions perpendicular to the galactic plane. Cosmological effects—principally redshift—might be invoked if the sources of gamma-ray bursts are extragalactic. Some caution is appropriate in interpreting the results, however, since

event rates are being compared between different detectors of somewhat uncertain apertures, efficiencies and energy ranges. Consequently there are errors in the integrated fluxes that are difficult to estimate. The Imperial College detectors appear to be sensitive enough to avoid limitations due to the transient nature of the bursts. This would only become serious if a substantial fraction of the bursts had timescales of 10 s or more, but there is at present no indication of this being so.

There are undoubtedly many other groups building or flying balloon-borne detectors to monitor weak gamma-ray bursts. It is hoped that these will confirm the first results and will eventually define the shape of the size distribution of weak gamma-ray bursts. The rare stronger bursts will probably only be available for study by satellite detectors. We are still no closer to a theoretical interpretation of gamma-ray bursts, although it is notable that the number of theories proposed to explain them is now lagging behind the number of detected events. □

Fish that change sex

from John R. Krebs

To a narrow-minded mammal the sex life of the Blue-headed Wrasse, a tropical reef fish, may seem odd. The Blue-headed Wrasse occurs in two colour phases: smaller fish are highly variable in colour and may be either male or female; while larger, older individuals are always blue and green with two vertical black stripes, and are invariably male. Some individuals go through the first (initial phase) colour pattern as a female and turn into a male when they reach the striking terminal colour phase. Other individuals go through both phases as a male. The transition from initial to terminal colour phase and the accompanying sex change can be achieved rapidly. If all the terminal phase males are taken away from an area of reef, initial phase males or females soon start to change colour and become terminal males.

Most of the fertilising of females is done by terminal males. They set up temporary territories at around midday at a specific spawning site on the reef. Here they wait for females to turn up, while the smaller initial phase males mill around in large numbers (often in hundreds). When females arrive at the spawning area they prefer to mate with the territorial terminal males, and a lucky male may mate up to 100 times a day. Initial phase males sometimes manage to fertilise a

NITROGEN fixation commonly occurs in bacteria only when the oxygen tension is low either because the nitrogen fixer is an obligate anaerobe such as *Clostridium* or because the aerobic organism, *Azotobacter* for example, has in addition to the normal ATP synthesising system, a very active electron transport chain that operates without conserving energy. This results in a micro-aerobic environment around the site of nitrogen fixation (Postgate, in *Biological nitrogen fixation*, edit. by Quispel, A., 1974) and brings about a respiratory protection of the nitrogenase.

The nitrogen-fixing blue-green algae have a particular problem since their photosynthesis leads to oxygen evolution; they may fix nitrogen only at low oxygen tensions and low light intensity (non-heterocystous forms) or they may segregate their nitrogenase into special compartments (the heterocysts) to which the fixation of nitrogen is normally confined. Only under artificial conditions of anaerobiosis do the ordinary vegetative cells of heterocystous blue greens have active nitrogenase.

Heterocysts are specialised in that they seem to lack the oxygen-evolving component of photosyn-

thesis, photosystem 2. They may also remove unwanted oxygen diffusing in from the environment by the use of an active respiratory chain. Thus a low oxygen tension could be maintained at the site of nitrogen fixation.

The absence of photosystem 2 has been deduced from the lack of accessory pigments, from the low yields of chlorophyll fluorescence and from the inability of heterocysts to fix carbon dioxide (Stewart, *A. Rev.*

Nitrogen, oxygen and manganese

from F. R. Whatley

Microbiol., 27, 283; 1973). The association of photosystem 2 with manganese has also been widely documented (for a review see Cheniae, *A. Rev. Pl. Physiol.*, 21, 467; 1970) although the evidence has always been indirect. Recently Tel-Or and Avron (*Proc. third int. Congress on Photosynthesis*, 569; 1974) reported isolation of a heat stable manganese-containing compound of small molecular weight which, when added back to depleted particles isolated from a blue-green alga (*Phormidium luridum*) restored oxygen evolving ability, thus

providing direct evidence of a role for manganese in O_2 evolution.

The lack of photosystem 2 in heterocysts in another blue-green alga (*Anabaena cylindrica*) is now further substantiated (Tel-Or and Stewart, *Nature*, 258, 715; 1975), following an examination of the distribution of ^{54}Mn between vegetative cells and heterocysts isolated from cultures grown with ^{54}Mn . This showed a correlation between the presence of ^{54}Mn , the Mn:chlorophyll ratio, and the ability of the isolated cells to evolve oxygen. The clear conclusion is that heterocysts of these nitrogen-fixing algae are deficient in manganese, that they lack a functional photosystem 2 and therefore cannot evolve oxygen. Heterocysts are thus specially adapted for nitrogen fixation. By contrast, the vegetative cells accumulate manganese and evolve oxygen but do not appear to fix nitrogen. It is of correlative interest (Anderson *et al. Biochem. biophys. Res. Commun.*, 17, 685; 1974) that fractions of isolated chloroplasts which have been depleted in photosystem 2 also become depleted in bound manganese whereas those enriched in photosystem 2 become enriched in manganese.

female's eggs by dashing into a territory at the last moment. More often, they chase an arriving female as a group and achieve a sort of piscine gang bang.

The facts are remarkable enough, but what is the evolutionary explanation for this strange reproductive system? Warner, Robertson and Leigh (*Science*, 190, 633-638; 1975) develop an idea of M. T. Ghiselin, who argued that natural selection will favour sex change whenever the reproductive potential of one sex increases rapidly with age while the other remains fairly constant. In this circumstance it will be advantageous to start life as the sex which does not benefit from being older (or to put it another way, does not suffer from being young) and switch to the other sex at a later stage. This is what seems to be happening in the Blue-headed Wrasse. Terminal males have an enormous reproductive potential, while initial males have a low mating success, so an individual can get the best of both worlds, by starting off as a female (young females do not suffer any penalty for youth) and switch to being a male at a later stage when it can successfully defend a mating territory. But why do some individuals go through the initial phase as a male and not a female? Warner *et al.* suggest that in large populations, where many females arrive simul-

taneously at the spawning area, the opportunities for an initial phase male to grab the odd fertilisation are high enough to make being a male in early life about as profitable as being a female. Their data show that the daily mating rates of an initial male and a female in a large population are about the same. In a small population, however, there are fewer chances for the initial males. There are rarely enough females at the spawning site to enable an initial male to steal a fertilisation. As this account would predict, initial males are much rarer in small populations than in large ones. Also, in other wrasse species that always occur in small groups, initial phase males are always rare.

If, as Warner *et al.* suggest, sex change is an optimal strategy when there is a big advantage in being old in one sex but not in the other, why don't mammals such as seals, deer and baboons, in which the older males monopolise females, change sex like the wrasse? The authors suggest that one or more of three factors may prevent them from doing so: the physiological cost of changing sex in an animal with internal fertilisation, the rigidity of the mammalian sex determination system, and the fact that in mammals competitive experience as well as size is important in determining the reproductive superi-

ority of older males. Obviously this experience would be lost if a male spent its youth as a member of the opposite sex. □

How old are cosmic rays?

from J. J. Quenby

WHILE recent progress in observational astrophysics has been rapid, the answer to the fundamental question as to the origin of cosmic radiation remains elusive. This flux of relativistic, charged particles populates the Galaxy with an energy density sufficient to influence significantly the dynamics of the whole system, but we do not know whether relatively near objects like the Crab Nebula or distant, energetic objects like strong radio galaxies constitute the prime source. An important clue would lie in an ability to 'read' the ^{10}Be 'clock' in the cosmic ray flux. The light elements Li, Be and B are rare in cosmic material and should not be present at the source of particle acceleration. By knowing production cross sections for the light elements in collisions between nucleons and atoms it is possible to calculate the amount of material through which the average

cosmic ray must have passed in order to produce the observed elemental abundance in the radiation. Then by finding the amount of radioactive ^{10}Be , which has a half-life for decay of 1.5×10^6 yr, relative to other light nuclei abundances, the actual cosmic ray age is deduced. If cosmic rays are not of Universal origin, that is to say, if they do not fill extra-galactic space as they do the Galaxy, then they must be confined either to the region of the galactic disk or galactic halo. The latter is an approximately spherical region, including the stellar disk, maintained by magnetic fields which trap charged particles and cause radio emission. By cosmic ray life we mean their time to diffuse or leak from the confinement region, although some are lost catastrophically by nuclear collision with the material of the trap. Present estimates allow a cosmic ray life of a few 10^6 years if confinement is to the disk, or alternatively a life in excess of 10^7 years if confinement is to the halo.

Satellite data on the ^{10}Be abundance obtained by Garcia-Munoz, Mason and Simpson of Chicago University (*Astrophys. J. Lett.*, **201**, L141-144; L145-148; 1975) suggests a halo rather than a disk confinement. These workers employed small charged particle telescopes mounted on the IMP-7 and IMP-8 satellites which sampled interplanetary conditions. Isotopes were separated to an accuracy of 0.4 atomic mass units by looking at energy loss in two solid state detectors and residual particle energy in a crystal scintillator. They found few or no ^{10}Be particles although ^7Be , ^9Be and ^{10}B events were detected.

If accepted, these interesting results will ease a related problem, concerned with the anisotropy in galactic arrival directions of the cosmic ray flux. Although an asymmetry of roughly 0.01% seems to be present, according to Imperial College measurements, the demands on the magnetic scattering process for a disk confinement model are severe. The short total path length requires a large amount of scattering to keep the anisotropy small and this in turn causes a large energy drain as the charged particles interact with the scattering centres. Halo confinement means less scattering and hence less energy drain. Alternatively the small anisotropy would be thought to imply a universal cosmic ray flux.

Although the Chicago group have certainly developed a high-quality experimental technique, some difficulties remain with the result. To date Balloon groups at Goddard Space Flight Center and the University of New Hampshire have also achieved isotopic resolution in the light element range, but they report ^{10}Be fluxes twice or four times those suggested by the satellite data. It

could be, however, that interactions above the balloon or magnetospheric processes have influenced the balloon results. Another point is that the lifetime deduced depends on assumptions as to the cosmic ray source spectra and these have to be obtained from trial models for the motion and interactions of cosmic rays in the confinement region. Furthermore, the final result clearly depends on the allowance made for solar modulation in the interplanetary medium. Diffusion and energy loss effects due to the interplanetary magnetic field tend to enhance the amount of ^{10}Be observed relative to neighbouring nuclei. At present we do not know whether the total cosmic ray flux at Earth is close to the interstellar value or as much as six times lower. If the modulation is small then even the Chicago ^{10}Be flux would be consistent with disk confinement and life of a few 10^6 yr for cosmic rays. Thus our final reading of the ^{10}Be 'clock' will depend on further studies to determine the scattering power of the interplanetary medium. A definitive answer probably requires a direct measurement of the interstellar cosmic ray flux, but this may not be achieved with planned missions in the ecliptic plane to Uranus and beyond. Indeed the lack of noticeable change in the cosmic ray intensity on the way out to Jupiter, as recorded by Pioneers 10 and 11, is one of the puzzles which illustrate our lack of understanding of modulation. Perhaps an off-ecliptic mission to solar polar latitudes where cosmic ray access is thought to be easier is the correct experiment for determining the ^{10}Be abundance. □

The Red Queen dethroned

from A. Hallam

IN recent years there has been an increasing tendency in some circles to investigate the evolutionary record of fossils in terms of general rules and processes without regard to specific causes operating on specific taxa. Van Valen (*Evol. Theory*, **1**, 1; 1973) has made a notable contribution in this respect by applying the survivorship curve technique of population biologists to the study of extinction rates for numerous fossil taxa. Van Valen claimed to demonstrate a general approximation to linearity in his curves, which are cumulative frequency distributions of taxonomic durations with logarithmic ordinates. This led him to propose a new evolutionary "law", which, in brief, states that within a relatively homogeneous higher taxon,

subtaxa tend to become extinct at a stochastically constant rate. As an explanation Van Valen put forward what he termed the Red Queen's hypothesis (because of the Lewis Carroll character who found it took all the running one can do to keep in the same place). In concise terms, the hypothesis states that increase in the momentary fitness of any set of species within a specific adaptive zone acts to reduce the fitness of all other species within that zone, in such a way as to cause the effective environment of all the species to deteriorate in a stochastically constant manner through time.

Van Valen's work has aroused considerable interest and provoked active discussion. Foin *et al.* (*Nature*, **257**, 515; 1975) criticise his use of survivorship curves because of the confusion between two quite different methods of analysis and because of the weakness of the analogy between cohorts of organisms born together and taxa which arose at different times. On the other hand Raup (*Paleobiology*, **1**, 82; 1975) generally approves of Van Valen's approach but suggests some methodological improvements; he does not attempt to decide whether or not the proposed law is correct. Another criticism of Foin and his coworkers is that the level of aggregation of the fossil data is so high that it is bound to eliminate all but the most extraordinary events, and apparently log linear survivorship curves are therefore inevitable. To this Van Valen replies that this averaging effect is a positive advantage since it eliminates "noise". He also attempts to clear up a conceptual confusion among his critics. He has never made the sweeping statement that the probability of extinction of a given taxon is independent of age, but argued instead that the mean extinction probability of a group is constant over long periods of time.

Salthe (*Paleobiology*, **1**, 356; 1975) is another who is unimpressed with Van Valen's "law". A stochastic approach to constancy of extinction rates merely signifies that there have been no systematic forces operating on the organisms in question, so that in fact there is nothing that requires explanation in any causal sense. The apparent constancy of extinction has, however, been thrown into question by the methodological analysis of Seposki (*Paleobiology*, **1**, 343; 1975). Seposki points out that the durations of taxa cited by Van Valen are in effect time interval estimates and these time intervals vary in length, approximating to a lognormal distribution. The resultant effect is to impart a systematic bias to the palaeontological data such that linearity, implying constancy of extinction rate, is approximated in the taxonomic survivorship curves. When

corrections are applied, it is estimated that, of Van Valen's 82 survivorship curves for extinct taxa, only 12% are definitely linear. Evidently Van Valen cannot win. Either his results show linearity, which is held to be biologically without significance, or most do not, in which case his "law" breaks down.

Although it begins to look as though the Red Queen has been toppled from her throne, the criticism directed at Van Valen's work should not be held to detract from an original, perceptive and imaginative attempt to glean more from the fossil record than a more conventional approach would allow. It has been stimulating in the best sense and may yet lead to important new advances in our understanding of evolution. That it appeared in the first issue of an obscure journal with limited circulation lends support to the view that if a paper is sufficiently interesting it will be widely read, regardless of where it was published. □

Strong absorption in ^{40}Ca – ^{40}Ca elastic scattering

from P. E. Hodgson

MANY studies of the elastic scattering of heavy ions have now been made, and it is usually found that the differential cross section has a diffraction structure that varies smoothly with the incident energy and is well described by the optical model.

A notable exception to this behaviour was found some years ago by Bromley *et al.* (*Phys. Rev. Lett.*, **19**, 369; 1967; *Phys. Rev. Lett.*, **20**, 175; 1968): the cross section for ^{16}O – ^{16}O elastic scattering shows a very irregular behaviour as a function of incident energy. This is in marked contrast to the corresponding data for ^{18}O – ^{18}O , which varies smoothly with energy as expected.

This early work, together with subsequent studies, showed that this notable difference in behaviour is determined by a balance between the strong absorption of the lower partial waves and the weaker absorption of the higher partial waves. For heavy ion collisions many features of classical behaviour remain, so that partial waves correspond to the interactions at different radial distances: the lower to interactions inside the nucleus and the outer to interactions in the nuclear surface and beyond. The scattering is thus determined by the absorbing part of the optical potential, and it was found that the observed behaviour could well be reproduced by allowing

the strength of the absorption to depend on the orbital angular momentum quantum number L , being strong for small L and weak for large L .

More physically, it was shown that the absorption is mainly due to the direct reactions strongly coupled to the entrance channel. When there are many of these, the nuclear surface is nearly opaque and one observes the small structureless cross section strongly decreasing with energy—behaviour typical of strong absorption. This is the case for ^{18}O – ^{18}O , which has two neutrons outside the stable ^{16}O core that can be excited and transferred in many ways, leading to many possible reaction channels.

The situation is quite different for ^{16}O – ^{16}O which is stable and difficult to excite and so absorbs weakly. Each partial wave makes its own particular contribution, giving the strongly-marked fluctuations in the cross section as a function of energy.

This explanation accounts for all the data in the oxygen region, but it is clearly desirable to confirm it by measurements on other nuclei. It is increasingly difficult to make measurements on heavier nuclei because the strong coulomb repulsion must be overcome before the nuclear interactions can take place, and this requires high incident energies.

The next lightest nucleus after ^{16}O that has a very stable doubly-closed shell structure is ^{40}Ca , and so it is desirable to make measurements of ^{40}Ca – ^{40}Ca scattering. This has recently been done by Doubré *et al.* (*Phys. Rev. Lett.*, **35**, 508; 1975) at Orsay in France, and some of their results are shown in the figure.

It is notable that, contrary to ex-

pectation, the cross sections fall smoothly with increasing energy, so characteristic of strong absorption. An optical model calculation with an absorption increasing with energy agrees well with the experimental data. The physical explanation of this result is not clear. The explanation used in the oxygen region fails for calcium, and it may be that the density of states in the appropriate region of excitation is sufficiently high in the calcium case for the required absorption to occur. More measurements are in progress to provide data for a more comprehensive study of these effects. □

Dominance and diversity

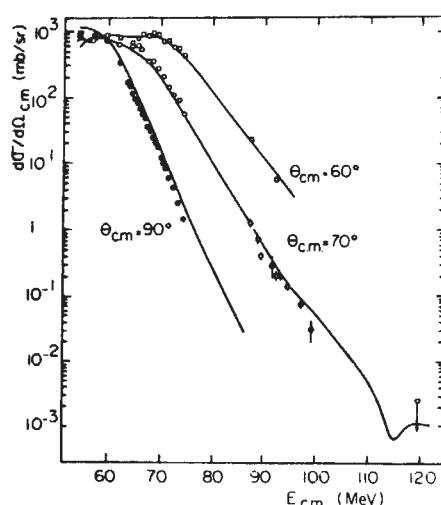
from Peter D. Moore

By far the most frequently used index of diversity in studies of plant communities is that derived from Shannon and Weaver's information theory, in which,

$$H' = -\sum p_i \ln p_i,$$

where p_i is the proportion of the total number of individuals (or biomass) belonging to the i th species. This index is a function of both species richness and evenness (the apportionment of individuals or biomass amongst species). The feature of plant communities to which the index responds most effectively is the presence of one or more dominant species. This results both from the consequential inequitable arrangement of biomass or numbers in the community and from the reduction in species number which often accompanies the assertion of dominance by certain members of the community. When one speaks, therefore, of high diversity in plant communities, one is often in fact referring to a lack of dominance.

Because of this it is unwise to assume that diversity changes as a consequence of a particular management practice, such as the abandonment of arable land, or the disturbance of equilibrium communities, can be easily predicted. Such unpredictability is a feature which has been demonstrated in two recent pieces of work in the United States concerned with precisely these situations. Tramer (*Ecology*, **56**, 905; 1975) has studied selected plots on abandoned agricultural land in Ohio over a period of four years and has attempted to document changes in plant species diversity. Numerically based diversity was highest in the second year after abandonment and subsequently fell to a lower level, while biomass-based diversity declined steadily over the four year period. This



Differential cross section for ^{40}Ca – ^{40}Ca elastic scattering at three angles as a function of energy and compared with optical model calculations.

process can be accounted for by the importance of biennial species in the second year and the subsequent assumption of dominance by the perennials *Aster pilosus* and *Solidago altissima* in the third and fourth year. There is certainly no indication that the popular conception of diversity increasing with succession applies here, but the observations are very short term from a successional point of view.

The dominance of large, perennial plant species in a community must, by its very definition, reduce species diversity. Successional developments involving invasion by such species can therefore be expected to show an accompanying decline in diversity. It is also true that the application of any additional stress, which reduces the performance of a potential dominant, serves to increase diversity, as has been demonstrated by Grime (*Nature*, **242**, 344; 1973) for both man-induced stresses and for natural environmental extremes. One such stress is that of predation, and the experiments of Paine (*Am. Nat.*, **103**, 65; 1966) showed how predation pressures serve to maintain diversity in intertidal communities. The same process operates in terrestrial plant communities grazed by herbivores (see Harper, *Brookhaven Symp. Biol.*, **22**, 48; 1969).

It is this complex interaction of grazing intensity and plant species diversity which provides an explanation for some of the data which Platt (*Ecol. Monogr.*, **45**, 285; 1975) has derived from his study of the recolonisation of areas of prairie disturbed by badgers. He selected for study the abandoned diggings of badgers which had been recovering for at least three years and compared their plant cover with that of the surrounding, undisturbed prairie. In virgin prairie areas, the badger mounds often had more species per unit area, but had a slightly lower overall diversity than their surroundings. This could be related to the important role played by *Andropogon gerardi* on the mounds, which was not observed to the same extent in surrounding areas. Overgrazed prairie regions bore a different plant community in which the grass *Poa pratensis* was dominant, at times accounting for 60% of the above-ground biomass. The diversity of such overgrazed prairie was found to be very much lower than that of virgin areas. Badger mounds in the overgrazed prairie, however, had as high a species diversity as those in virgin areas and were therefore considerably more diverse than surrounding communities. This was the consequence of a reduction in the importance of *Poa pratensis* (to about 20% of the biomass) and also a higher species richness.

Once again the question of successional development is seen to be

irrelevant to the determination of overall plant community diversity, except in that it may relate to the development of dominance. Any factor, whether related to succession or imposed by some external stress, which reduces the establishment or growth rate of potentially dominant, perennial plants, will enhance the species diversity of the community. □

Hydrothermal activity off-ridge?

from Peter J. Smith

THERE is now considerable evidence to suggest that metalliferous oceanic sediments are generally the result of hydrothermal activity. It is implicit in the close association of most such sediments with submarine volcanism, for example, that the added metals are precipitated from metal-rich solutions produced by the reaction of seawater with cooling magma. In practice, the association with submarine volcanism means that many enriched sediments have been observed in the vicinity of active oceanic ridge crests, for it is there that the bulk of the Earth's volcanic activity occurs. But there are exceptions; metal-rich deposits have been found in, for example, the Bauer Deep. What, then, is the origin of these off-ridge enriched sediments?

As the Bauer Deep lies immediately between the extinct Galapagos Rise and the active East Pacific Rise, it can hardly be said to be totally removed from a ridge environment. One possibility therefore is that the metal-rich deposits (as indicated by high transition metal to aluminium ratios) were formed along the East Pacific Rise by hydrothermal processes and subsequently carried across the rise flank to the Deep. But as Dymond and Veeh (*Earth planet. Sci. Lett.*, **28**, 13; 1975) now point out, there are other possible explanations. There could be a single, wide hydrothermal zone, centred on the East Pacific Rise and encompassing the Bauer Deep, within which the metal/aluminium deposition varies. Alternatively, the Rise and the Deep could be associated with separate hydrothermal systems. Or the Rise crest sediments could be hydrothermal and the Deep sediments largely authigenic.

In an attempt to determine which, if any, of these models is the correct one, Dymond and Veeh have analysed metal accumulation rates in cores from the East Pacific Rise crest, the flanks of the Rise and the Bauer Deep. The role of aluminium in marine environments is poorly understood, and so the significance of variations in the transition metal/aluminium ratio itself is difficult to assess. Nevertheless, the separate

patterns of accumulation rates of Fe, Mn and Al across the Rise-Deep zone seem to be inconsistent with the patterns expected from a wide hydrothermal region. The observed patterns all peak in the Deep as well as at the Rise, suggesting that the idea of a slow decrease in hydrothermal activity from the Rise outwards cannot be valid.

The peaking across the Deep also rules out a more rapid fall of hydrothermal activity away from the Rise in conjunction with completely authigenic precipitation in the vicinity of the Deep. Estimates made by Dymond and Veeh suggest that 30–50% of the manganese and all of the nickel in the Bauer Deep could in fact be deposited by authigenic processes; and given the errors on these estimates, this means that an authigenic origin for most of the minor transition metals cannot be ruled out. But the same cannot be said for iron. The amount of iron present is such as to eliminate authigenic activity as a primary source, even if it is allowed that some of the iron could be detritus from land-derived clays and volcanic debris.

This then leaves two models: the double (Rise and Deep) hydrothermal source and the single (Rise) source with transport of metal-rich sediment to the Deep. Unfortunately, since the two models give similar distributions of metals, it is not possible to distinguish between them on the basis of Fe and Al accumulation rate data alone. Although there is a maximum in metal accumulation over the Deep, there is apparently no comparable maximum on the opposite side of the Rise. This would seem to argue against a single source with transport, except that preferential transport of material away from the Rise in one direction cannot be ruled out (especially as knowledge of the bottom currents in the area is poor).

Then again, Anderson and Halunen (*Nature*, **251**, 473; 1974) have argued in favour of hydrothermal processes in the Bauer Deep on the basis of heat flow data, suggesting that such processes could have been initiated when spreading moved from the Galapagos Rise to the East Pacific Rise about 6.5 Myr ago. On the other hand, one of the Deep cores examined by Dymond and Veeh is probably about 20 Myr old; and persistent hydrothermal activity since this core was formed seems unlikely.

There is thus evidence (that given above and more) on both sides. Moreover, there are data suggesting that neither model is entirely correct. There are, for example, chemical differences between East Pacific Rise and Bauer Deep sediments, although many, if not all, of these could be explained on the basis of simultaneous authigenic depo-

sition in the Deep. For the time being the detailed picture remains unclear. What is certain, however, is that the rate of accumulation of metals in the Bauer Deep is an order of magnitude lower than that on the East Pacific Rise. □

"Turn-coat" trypanosomes undressed

from J. R. Baker

It has been known for some time that the tsetse-transmitted trypanosomes of Africa (including *Trypanosoma brucei*, which causes human sleeping sickness) have, at certain times in their life cycle, a 12–15 nm thick "coat" outside their unit plasma membrane. Mainly circumstantial evidence strongly suggested that this coat contained antigens presented to the vertebrate host which, by being structurally changed every time the host produced a specific antibody, contributed to the parasites' survival and, hence, the eventual death of the host (Vickerman, *Ciba Symposium*, **25**, 53; 1974) and also to our inability to produce an effective anti-trypanosomiasis vaccine.

A few years ago, Wright and Hales in Toronto (*J. Parasit.*, **56**, 671; 1970) suggested that the coat consisted of carbohydrate and protein, and work by them and Lumsden (*J. Cell Sci.*, **6**, 285; 1970) led to the idea that the trypanosomes may cast off old antigen by releasing long tubular threads of encoated plasma membrane (though the natural production of these threads *in vivo* has never been conclusively demonstrated). In Basel, Steiger produced evidence that new coat was produced by the trypanosomes' Golgi-derived reticulum (*Acta trop.*, **28**, 341; 1971 and **30**, 64; 1973). This study took an exciting step forward three years ago when Cross reported to the 13th Trypanosomiasis Seminar in London the isolation of a single glycoprotein apparently representing dissociated coat (*Trans. R. Soc. trop. Med. Hyg.*, **67**, 261; 1973). Significantly, the putative coat proteins prepared from different clones of one strain of *T. brucei* differed in composition. This supported the view that coat protein is the variant antigen, an idea now further strengthened by Cross's latest discovery that mice immunised with purified coat glycoprotein (molecular weight $65,000 \pm 3,000$) are protected against subsequent infection with living trypanosomes of the same, but not of other, clones (*Parasitology*, **71**, 393; 1975). One of the major remaining problems concerns the cellular control of this antigen switch: it is insufficiently random to be explained easily by mutation and its occurrence within a

clone appears to preclude its resulting by selection from a pre-existent mixed population. Current thinking—stemming, incidentally, from work by Ehrlich and others in 1909 (*Z. Immunitätsforsch.*, **3**, 296; 1909)—favours a rather vague concept of induction by antibody. Cross's work may well prove to be an important key in the eventual unlocking of this mystery which, in turn, could conceivably lead to successful vaccination against sleeping sickness. □



A hundred years ago

A REMARKABLY valuable discussion by M. Belgrand, of the inundations of the Garonne, viewed specially in connection with the heavy rains which fell over France from the 21st to the 24th of June last, has been appearing at intervals for the past fortnight in the *Bulletin International* of the Paris Observatory. It is pointed out, from the dates of their occurrence, that the inundations of the southern portion of the basin of the Garonne which slants from the Pyrénées, have nearly always occurred in spring or early summer, and at the same dates either no floods at all, or comparatively unimportant floods, were experienced in the northern portion of the basin which slopes down from the Cevennes and central plateaux of France. It is to be noted that it is just at this season that the rainfall of the southern portion of France attains its annual maximum, and the nearer to the Pyrénées the more decidedly is the May–June maximum marked, and that the melting of the snows which have accumulated on the Pyrénées during the winter months proceeds most rapidly. On the other hand, it is shown that the great inundations of the northern portion of the basin occur generally during the cold months of the year, and that at the time of their occurrence there have been no corresponding great floods at Toulouse, in the southern portion of the basin. It is during the cold season that the rainfall reaches its annual maximum on leaving the slopes of the Pyrénées and advancing northwards over the basins of the Tarn, Lot, and Dordogne. The disastrous inundation of June, 1875, was in accordance with the experience of previous floods in the south of France. As a great flood it was limited to the river courses sloping down from the Pyrénées; and the nearest approach to a great flood elsewhere was in the basin of the Arout, the most southern tributary of the Tarn, and it was the flood of this tributary which occasioned almost the whole of the flood of the Tarn. At such places as Auch, situated in a narrow valley, and where, consequently, the drainage area is small, the inundation was much less disastrous than at Toulouse and places similarly situated near the confluence of large affluents draining a wide extent of the country.

from *Nature*, **13**, January 6, 197, 1876

Oscillating universe bounces back

from John Gribbin

THE biggest problem with the Big Bang theory of the origin of the Universe is philosophical—perhaps even theological—what was there before the bang? This problem alone was sufficient to give a great initial impetus to the Steady State theory; but with that theory now sadly in conflict with the observations, the best way round this initial difficulty is provided by a model in which the Universe expands from a singularity, collapses back again, and repeats the cycle indefinitely. Such models have been known since the earliest development of relativistic cosmologies in this century, but have been favoured with a revival of interest in recent years. The latest study provides some intriguing agreements with observation of the real Universe, but also poses some new puzzles (Landsberg and Park, *Proc. R. Soc. Lond.*, **A346**, 485–495; 1975).

Landsberg and Park consider a model universe composed of gas and radiation which are 'lowed to interact; this incorporates features of standard cosmology (taking account of gravitation) and of statistical mechanics (which does not take account of gravitation). The resulting hybrid is, they suggest "too simple to be realistic", but does go a step further than the standard dynamic models of traditional cosmology. Both the gas and the radiation (assumed black body) are uniform, which is as good an approximation as in any other cosmology, where such incidentals as galaxies are generally taken as 'test particles' of no great account.

The first important result which emerges from this incorporation of thermodynamics into cosmology is that during both the expanding and contracting phases of the oscillating model entropy increases, so that the "arrow of time" is constant. To follow the repeated 'bounce' through the initial/final singularity Landsberg and Park cheat a little by simply reversing the collapse at very great density, so that it becomes an expansion with the same speed. This may not be entirely valid, since it carries over information from one cycle to the next, but because the collapse phase proceeds more rapidly than the preceding expansion, this means that successive cycles of the oscillation start out faster and faster, expanding to greater and greater radii before they collapse in their turn, with each cycle taking longer than the last. This behaviour is in line with that of earlier oscillating models, but even

though it always happens with the models they have calculated, Landsberg and Park cannot prove that it must happen.

Such behaviour could explain why our Universe seems to be so close to the dividing line between continued infinite expansion and just turning back into collapse at some future time. In the Landsberg-Park cosmology, successive cycles of expansion/collapse bring the model ever closer to this dividing line, and the closeness of our Universe to the "just bound" condition might suggest that it has already been through many cycles. So, like the best theories, this one makes a firm prediction: future observations should show that the Universe is indeed just bound, and not just unbound.

But there are two snags with the theory. First, if successive cycles expand to bigger and bigger radii, running the calculation backwards would suggest that the bounce itself started in some infinitesimal hiccup some large number of cycles ago, so we still have an origin problem but in a different form. Second, and perhaps more serious, if the closeness of the Universe to the "just bound" condition means that it has been through many cycles then there has been ample opportunity for entropy to increase following the constant thermodynamic arrow of time of the model. Yet the most obvious feature of our Universe is its low entropy; as Hermann Bondi has said "thermodynamic properties tend to be very deep and significant: the fact that our night sky is very black, with very bright points, the stars, in it, may be the profoundest piece of knowledge of the universe that we have". But these, of course, are just the kind of questions that a more sophisticated version of this simple model might be expected to tackle. □

Rotation of planetary atmospheres

from a Correspondent

A meeting of the Royal Astronomical Society, Institute of Physics and the Royal Meteorological Society, held in London on November 28, 1975, provided interdisciplinary discussions of the atmospheric motions at all atmospheric levels.

STUDIES of atmospheric motions concern scientists from various backgrounds, whose research interests generally confine them to a small region of the entire atmosphere. Meteorologists, for example, are concerned with

the lowest levels which constitute the troposphere and stratosphere which extend from the surface to about 35 km for their studies of weather and climate; aeronomers are interested in the physics, chemistry and motions of the remaining portions of the neutral atmosphere, the stratosphere, mesosphere and thermosphere, which extend to about 100 km above the surface; while a third group study the ionised layers of the upper atmosphere. It may be scientifically convenient to partition the atmosphere in this way according to the dominant physical processes that occur. It is, however, of fundamental importance that we now study the whole atmosphere, and consider the energy transfers between these regions, so that we may determine whether external effects such as solar activity or stratospheric compositional changes could have any effect upon our climate in the troposphere.

By tracking orbiting satellites for more than a decade, D. King-Hele (Royal Aircraft Establishment, Farnborough) has found that Earth's atmosphere above about 100 km moves faster than the solid body of the planet. This is called "super rotation". The magnitude of the motion is highly variable, with altitude, latitude and time, although the largest value measured so far is about 160 m s^{-1} at an altitude of 240 km from tracking the COSMOS 344 satellite. The normal rotation at these levels would imply motions of 100 m s^{-1} . The Explorer 1 satellite has been tracked through two solar maxima, and suggested that the rotation rate decreased with a corresponding decrease in solar activity. Subrotational motions ($\leq 100 \text{ m s}^{-1}$) have so far been found only in the afternoon period at these altitudes. H. Rishbeth (SRC Appleton Laboratory, Slough) indicated that there is inadequate information to construct a theoretical model in an attempt to explain these motions. Unlike the winds in the lower atmosphere, they were not in geostrophic balance at these levels. He indicated that the driving mechanisms would differ on a local basis (for example, the polar cap, auroral zone, equatorial latitudes) and an integrated measurement/theoretical programme was urgently required. The deposition of interplanetary matter, in particular meteoroids, has often been thought to be a possible additional heating source of the upper atmosphere. D. W. Hughes (University of Sheffield) showed however, that there is insufficient material to modify the upper atmospheric motions in any significant manner.

In the stratosphere, wave motions play a major role in modifying the atmospheric motions as discussed by

D. G. Andrews (University College, London; Meteorological Office). The equatorial region has been found to undergo a quasi-biennial oscillation (QBO) in which, in a period of about 26 months, easterly motions become reversed into westerly motions. He suggested that Kelvin waves with a 15-d period could account for the easterly flow, while a Rossby gravity wave with a 4-d period could be responsible for the westerly flow. Andrews also thought that tropospheric changes may influence these stratospheric motions through the vertical propagation of energy. A. White (Imperial College, London) discussed the importance of baroclinic eddies in the transfer of troposphere energy. He showed that the structure of the flow was sensitive to the pole-to-equator temperature gradient and that changes in this parameter would modify the flow, producing a different climatology. White's studies indicated that simple models may be constructed to study climatic change over time intervals of several decades, through the parameterisation of the heat transfer effects of these eddies.

Other planetary atmospheres exhibit super rotation features, whose understanding could assist in determining the dominant driving mechanisms of the corresponding features of the Earth's atmosphere. R. A. Plumb (Meteorological Office) discussed the rotation of the Venusian stratosphere which moves sixty times faster than the solid body of the planet, producing zonal winds of about 100 m s^{-1} . The motion is generated by the upward momentum transport by the thermal tide in the Venusian stratosphere, driven by solar energy absorbed by the opaque clouds whose tops occur at about 200 mbar. Similar thermal forcing could not occur in the Earth's atmosphere, since most of the solar radiation is absorbed at the surface. Jupiter and Saturn also exhibit equatorial jets with zonal velocities of about 100 and 400 m s^{-1} respectively. R. Hide (Meteorological Office) stated that currently available theories were unable to account for these features.

This meeting emphasised the importance of the transfer of energy between atmospheric regions in explaining many of the observed motions. A more detailed understanding of the atmospheric dynamics requires further observations at all levels, which for the Earth's atmosphere is possible through the presently planned satellite and rocket programme as D. Rees (University College London) described. We may then expect further tripartite meetings of this type which are an invaluable forum for discussing the motions of planetary atmospheres. □

articles

High resolution observations of NGC1275 with a four-element intercontinental radio interferometer

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The radio nucleus of NGC1275 (3C84) is found to consist of three apparently stationary centres of emission extending over 0.006'' (3 pc) along position angle -9° . One or more of these centres have changed their intensity or size in the last 3 yr. A preferential direction of alignment of both the small scale and large scale radio features as well as the optical features suggests a common cause of alignment operating over linear distances from a few pc to > 100 kpc.

NGC1275 is an active radio galaxy and is one of the strongest extragalactic radio sources at centimetre wavelengths. The radio nucleus has been previously studied by very long baseline interferometer techniques¹⁻⁵, and has been shown to have a complex angular structure $\sim 0.006''$ in extent and elongated along position angle $\sim -10^\circ$. Because the radio nucleus has a relatively large angular size and a high brightness, it is uniquely suited for detailed high-resolution observations. The earlier measurements, however, had an insufficient number of antenna pairs and insufficient resolution to map the complex structure in any detail.

Intercontinental interferometer system

We report here new observations made at epoch 1974.5 with a 4-element intercontinental interferometer system which gave six simultaneous independent interferometers. The

observing frequency was 10,650 MHz ($\lambda = 2.8$ cm) giving a maximum baseline of $290 \times 10^6 \lambda$. This was sufficient to resolve a Gaussian source of $0.00025''$. The polarisation was left-circular.

The elements of the interferometer are described in Table 1 and the diurnal track of each interferometer in the Fourier transform (u, v) plane is illustrated in Fig. 1. Data were recorded at each station on an NRAO Mk II VLB terminal, and the tapes were correlated on the NRAO processor⁶. A description of the further averaging of the data, the calibration of the fringe amplitude and the experimental errors has been given elsewhere⁷.

Derivation of source structure

The observed data are shown in Fig. 2 in the form of plots of the fringe amplitude as a function of the interferometer hour angle. (The interferometer hour angle (IHA) is defined as the hour angle of the source measured from the meridian of the equatorial projection of the baseline.) The location in the (u, v) plane of each of the maxima and minima in the visibility function is indicated in Fig. 1.

The separation and direction of lines joining corresponding maxima and minima of the visibility function demonstrate the multiplicity of components and give the approximate extent of the source and the direction of elongation. In the inner part of the (u, v) plane the spacing corresponds to a total separation of about $0.006''$ between the outer major features; the somewhat closer spacing of the extrema on the longer baselines requires weak compact features separated by up to $0.01''$. The

Table 1 Interferometer elements

Location*	Diameter (m)	System noise temperature (K)	Frequency standard	Institution
Effelsberg, W. Germany	100	300	Rb	MPiR
Green Bank, W. Virginia, USA	43	120	Maser	NRAO
Fort Davis, Texas, USA	26	300	Rb**	HRAS
Big Pine, California, USA	40	225	Rb**	OVRO

*The geographic location of the stations and the baseline lengths have been given in ref. 7.

**A quartz oscillator was used to reduce the short term noise from the rubidium standard.

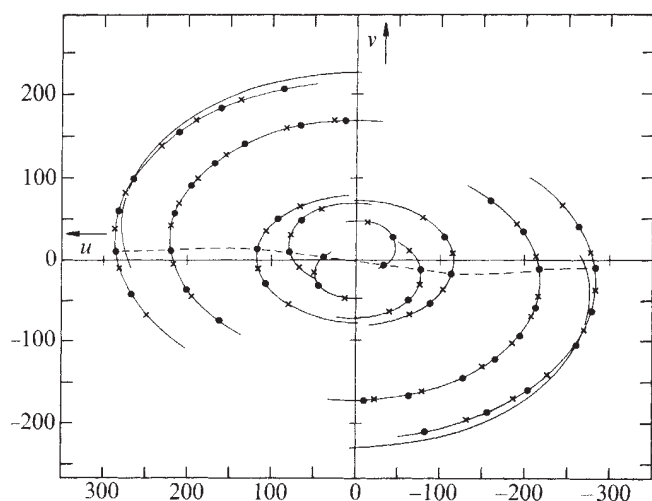


Fig. 1 Diurnal tracks for the six interferometers in the (u, v) plane. The units in u and v are in $\lambda \times 10^6$. Maxima and minima in the fringe visibility are shown by filled circles and crosses, respectively, except on one track (MPIR-HRAS), where the data are sparse. The line joining the principal maxima is shown dashed.

decrease in fringe amplitude along the line of the principal maximum (the line through the origin) shows that the size of the source along the minor axis is about $0.0015''$. As can be seen from Fig. 2, the maximum fringe amplitude on the three longer baselines is only $\sim 10\%$ of the total flux density of 58.4 Jy, while on the shortest baseline the fringe visibility reaches 90% . Most of the emission therefore originates in components larger than $0.001''$. The rapid changes in fringe amplitude with hour angle observed on the trans-Atlantic baselines are real and the data have been confirmed by repeated observations.

A model describing the source brightness distribution was derived as follows: first, the overall extent and elongation of the source was determined by inspection of Fig. 2. Next, the brightness distribution was approximated by a model consisting of 10 elliptical Gaussian components. The parameters of each of these components were found by an iterative procedure which minimised the r.m.s. deviations between the observed data and the visibility function of the model. This preliminary model was then decomposed into a grid of point sources spaced $0.0002''$ apart. The amplitude at each grid point was varied by another iterative procedure which further reduced the r.m.s. deviations between the observed visibility function and that of the model. Finally, the resulting grid was smoothed with a Gaussian beam having a FWHM of $0.0004''$. The resulting map of the radio structure of the NGC1275 nucleus is shown in Fig. 3, and the calculated fringe amplitudes of the unsmoothed model as a function of the IHA are shown in Fig. 2, together with the experimental data.

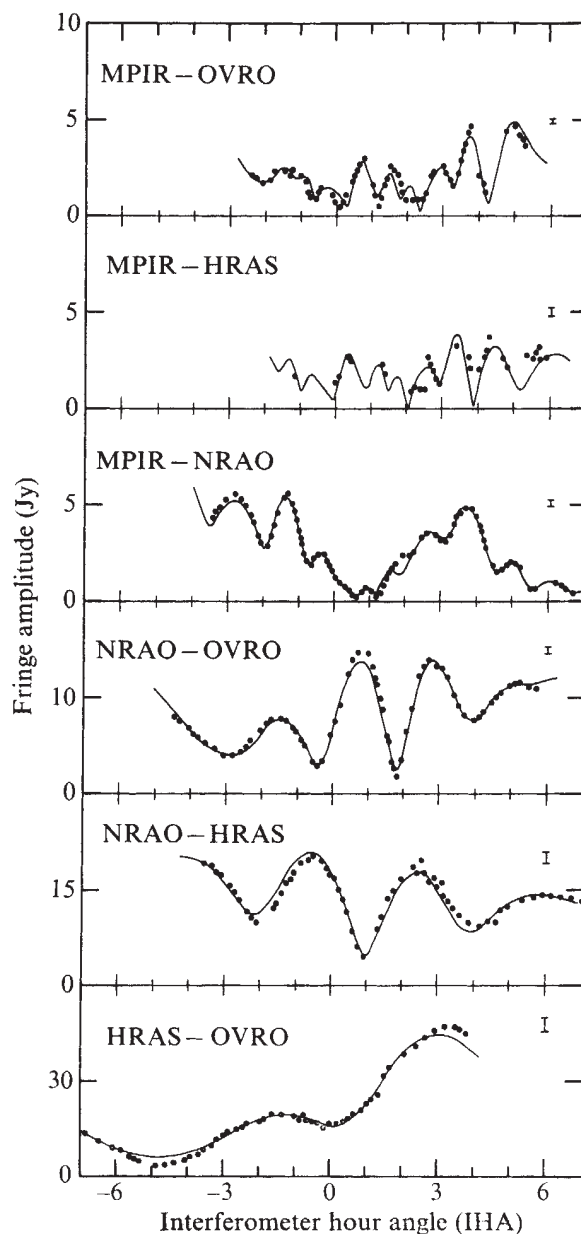
We emphasise that the map shown in Fig. 3 is based on fringe amplitudes alone, since we did not retain the phase information necessary to perform a Fourier inversion of the data. Models derived in this way are not unique; in particular, there is an ambiguity in the position angle of 180° . Also, the coverage of the (u, v) plane is incomplete, and even with phase information this would have led to sidelobes in the reconstructed map. To investigate the uniqueness of our model, we have used the iterative grid procedure starting from six-component and two-component models, rather than the ten components used to produce the map shown in Fig. 3. Each of these approaches gave very similar final maps. Moreover, four of the authors have independently derived a map using multi-component Gaussian models and different modelling procedures, and again these maps agree in their main features.

This is perhaps not surprising since the complexity of the

fringe visibility function, coupled with the restriction that the brightness should everywhere be positive, seems to provide constraints which facilitate the reconstruction of the main features of the brightness distribution even in the absence of phase data. It is always possible to find a more complex geometry which gives an even better fit, but the data do not justify more complex models. We believe, however, that the data do require a model of the complexity of that shown in Fig. 3. We note that the evidence for fine structure on a scale $\lesssim 0.001''$ comes primarily from the trans-Atlantic baselines. A much simpler model, consisting of five Gaussian components, fits the data from the three shortest baselines adequately, and reproduces the trend of the data from the longer baselines with deviations of only a few Jy.

As a further comparison, we have convolved our map with

Fig. 2 The fringe amplitude as a function of IHA for the six interferometers. The experimental data are shown as filled circles. The experimental errors for each interferometer are indicated by error bars at the right hand side of each plot. These are the errors corresponding to the largest fringe amplitude observed; errors near the minima of the fringe visibility are smaller than the size of the plotted symbols. The computed fringe amplitudes for the model structure are shown as solid curves.



a fan beam to produce a one-dimensional strip scan along the major axis. We find that this agrees qualitatively with the one-dimensional brightness distribution which has been derived³ from amplitude and phase-closure data obtained at 3.8 cm at epoch 1973.7.

Nature of radio source

The major features of the radio nucleus of NGC1275 seen in Fig. 3 are the three areas of high brightness. The separation between the peaks of the two outer regions is $0.006''$ and the overall extent along the major axis down to the 10% contour level is $0.01''$. The centres of the three major peaks lie nearly on a straight line in position angle -9° . In this sense the structure is 'colinear' although there are clearly significant deviations from a simple linear structure. The size (FWHM) along the minor axis is $\sim 0.0015''$. The brightest feature is located near the centre of the source and has a peak brightness temperature of about 10^{11} K on the smoothed map. Observations of still higher resolution will be required to study this compact region.

The redshift of NGC1275 is 0.018, corresponding to a distance of 110 Mpc ($H = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$), so that $0.001''$ is equivalent to a linear distance of 0.5 pc. The separation of the two major outer components is then 3 pc, and a significant part of the emission from the central component originates from a region smaller than 0.1 pc.

In Fig. 4 we have plotted the radio-frequency spectrum of the source 3C84 between 20 MHz and 100 GHz using the data taken from the literature as indicated. The spectrum can be broken down into three parts^{8,9}, associated with structure on different angular scales:

(A) A high frequency spectral component associated with the radio structure discussed here. This component becomes self-absorbed below about 5 GHz. At 10.65 GHz the measured total flux density was 58.4 Jy. Extrapolation of the two components B and C indicates that they contribute about 1.5 Jy at 10.65 GHz and our model of component A accounts for all the remaining flux density.

(B) A decimetre wavelength spectral component which becomes self-absorbed below 500 MHz. Purcell (personal communication) has studied this component with the NRAO-HRAS-OVRO interferometer at 609 MHz. The structure is elongated at a position angle near 0° , and is probably double with a component separation of about $0.05''$ (25 pc). The available data do not allow the relative positions of component A and the double source making up component B to be determined accurately. The centroids of the two components must, however, lie within a few arc seconds of each other.

(C) A component with a 'normal' power-law spectrum. This extended feature has been studied¹⁵ with the Westerbork array at 1,420 MHz. It consists of a $30''$ (15 kpc) source coincident with the galaxy and a larger, $5'$ source (150 kpc), both of which are elongated roughly along position angle -10° .

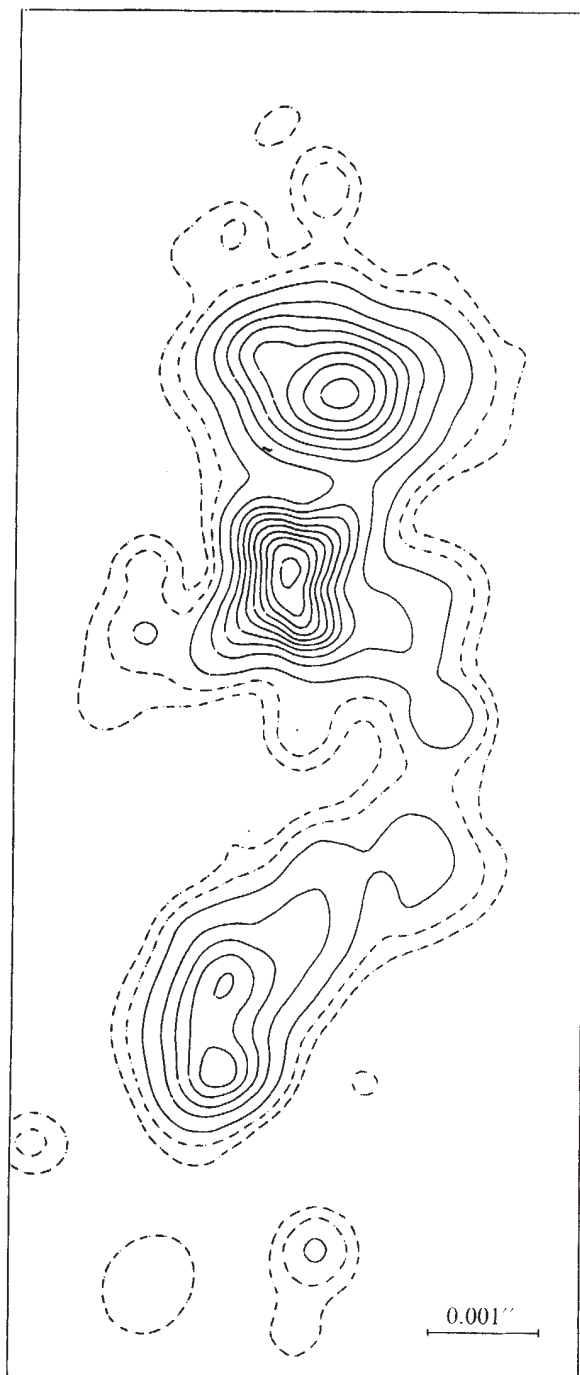
Each of the components A, B, and C is elongated in essentially the same direction. Moreover, the H α filaments, which extend¹⁶ more than $140''$ from the centre of the galaxy, also have their greatest extent in this direction. This suggests a common mechanism of alignment which is effective over linear scales ranging from a few pc in the dense nucleus of the galaxy to more than 100 kpc in intergalactic space.

The 150 kpc component must be at least 5×10^5 yr old and is probably 10–100 times older. Component A, on the other hand, is probably not more than 20 yr old. Although the earliest observations¹⁷ of the flux density of 3C84 at 10.7 GHz were made in 1966, measurements at 8 GHz (ref. 18) go back to 1960 at which time the flux density was only about 10 Jy. At the time of our measurements, however, the flux density at 8 GHz had increased to 55 Jy (Blankenship, personal communication), so that most of the present component A did not exist before 1960. Numerous observations at centimetre wavelengths since 1965 show that the flux density of component A increased in an approximately linear manner until

1973 and has remained roughly constant since that time (for example, refs 5, 19, 20).

Unfortunately, we have high resolution observations beginning only in 1972, so that we cannot trace the development of component A in detail. Previous interferometer data⁵ taken at 10.7 GHz with an angular resolution of about $0.001''$, made with the NRAO-HRAS-OVRO antennae at the epochs 1972 April, 1972 October and 1973 March, show that the maxima and minima in the fringe visibility have remained at nearly constant positions in the (u,v) plane, while the amplitudes of the extrema have varied considerably over this time.

Fig. 3 The structure of the radio nucleus of NGC1275, derived as described in the text. Contours are given in 10% intervals of the maximum brightness down to the 10% level; the 5 and 2.5% levels are shown dashed. The scale in the two coordinates is indicated by the $0.001''$ bar in the lower right corner. North is at the top and East at the left, but there is a 180° ambiguity in the orientation of the map (see text).



This implies that the overall dimensions of the source and the relative locations of the three bright condensations have not changed significantly, but their intensity and/or size has varied during this time. Thus the nucleus of NGC1275 seems to contain three apparently stationary centres, separated by

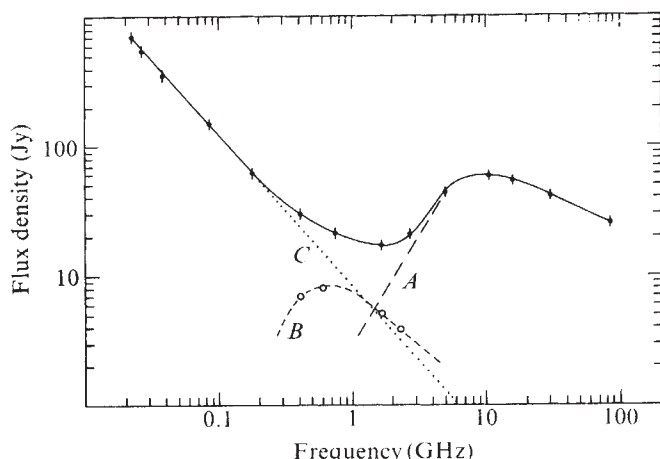


Fig. 4 The radio spectrum of NGC1275. Above 1 GHz, the epoch is that of the present measurements (1974.5). Total flux density data below 1.65 GHz are taken from Ryle and Windram (ref. 8); at 1.65 GHz from unpublished data obtained by Webber, Denoyer and Swenson; above this frequency from unpublished measurements made by the authors and A. Niell. The separation into spectral components *A* (—), *B* (---) and *C* (···) is based on interferometer data at 408 MHz and 1.4 GHz (ref. 10); 448 MHz (ref. 11); 609 MHz (Purcell, personal communication); 1.4, 2.7 and 5 GHz (ref. 12); 1.65 GHz (ref. 13) and 2.3 GHz (ref. 14, and unpublished data). In making the separation, we have assumed that components *B* and *C* have not varied during the period 1969 to 1974.

a few pc and lying nearly along a straight line; at least one of these centres has been active in the past three years. This is in contrast with the quasar 3C273 and the radio galaxy 3C120, where the location of the extrema changes rapidly⁵, suggesting rapid movements of the radiating components.

Repeated observations with multi-element intercontinental interferometer systems are necessary to study the details of the time variations in the nucleus of NGC1275. This would give unprecedented insight into the powerful energy source located in the nuclei of galaxies and specifically into the mechanism responsible for the origin of extragalactic radio sources.

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The structure of the protein disk of tobacco mosaic virus to 5 Å resolution

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An electron density map of the TMV disk at 5 Å resolution has been obtained using isomorphous replacement and non-crystallographic symmetry. The polypeptide chain can be traced with little ambiguity. The axial contacts between protein subunits are unlike those in the virus, the disk being a more open structure apparently designed for rapid interaction with the RNA.

THE disk of tobacco mosaic virus (TMV) protein is crucial in the assembly of the virus from its RNA and protein. It is composed of two rings each containing 17 protein subunits¹, both layers facing the same way². The disk has long been known as one of the polymorphic forms of TMV protein³, but it was only recently shown to be the dominant aggregate at neutral pH and moderate ionic strength⁴, essential for nucleation of the virus assembly, and to contribute to the rapid elongation of the nucleoprotein rod^{5,6}. The interaction of the disk with a special sequence on the RNA also provides a mechanism for recognition by the protein of its homologous RNA⁵.

The X-ray diffraction study of the disk was begun before its relevance to virus assembly had been established, when crystals were obtained during systematic attempts to crystallise small aggregates of the subunits¹. These earlier X-ray studies, leading to a low resolution electron density map, have been described^{7–9}. Because of the large size of the unit cell, which has a whole disk of molecular weight 600,000 as its asymmetric unit, the extension to high resolution posed a formidable technical problem, which was overcome only by the development in this laboratory of an automated camera, together with a computer-linked densitometer and associated computer programs for processing the hundreds of thousands of X-ray intensities involved^{10–12}. Here we describe the extension of the X-ray work to 5 Å resolution.

The structure has been solved by the method of isomorphous replacement. Only two heavy-atom derivatives were used, but good phases were nevertheless obtained by using the 17-fold rotational symmetry of the disk; this non-crystallographic axis gives rise to redundant information in the X-ray data which can be exploited in phase determination. The validity of the final electron density map is demonstrated by the striking similarity between the two

rings of the disk, which are crystallographically independent and whose similarity was not assumed at any stage. The map is sufficiently clear for the course of the polypeptide chain to be traced with little ambiguity.

X-ray data collection

The preparation and crystallisation of the virus protein has been described by Leberman *et al.*⁷ The crystals are orthorhombic, space group $P2_12_1$, with unit cell $a=228$ Å, $b=224$ Å and $c=174$ Å. The disks stack in pairs related by the crystal dyad⁸ with their 17-fold rotation axis approximately parallel to c .

There are 39,000 unique reflections to 5 Å, and a complete set of data, allowing for scaling overlaps, requires the recording of about 200,000 reflections. X-ray intensities were first collected from crystals of the native protein and of a covalently-bound methylmercury derivative⁷. Subsequently, to check and improve the phasing, data were also collected from a $(\text{AuCl})_2^{3-}$ derivative, but this proved to have low occupancy.

X-ray photographs were taken on an Arndt-Wonacott oscillation camera¹⁰ using small contiguous rotations, about the b axis, of approximately 1° per film pack. The total rotation angle required was 90° , necessitating several crystals for each set of data. Reflections occurring at the

Table 1 Statistics of data collection to 5 Å

	Native	Mercury	Gold
Total reflections measured	181,000	198,000	118,000
No. of split reflections	57,000	73,000	42,000
No. of independent reflections	38,800	39,100	37,600
$R_{\text{sym}} = \sum F_o - F_c / \sum F_o$	0.14	0.10	0.13
$R_{\text{sym}}, 50\%$ strongest reflections	0.08	0.06	0.07
Mean isomorphous difference $\langle F_{\text{nat}} - F_{\text{der}} \rangle / \langle F_{\text{der}} \rangle$	—	0.30	0.19

The films were scaled together by means of symmetry-related reflections recorded on different films and a symmetry R factor was computed for each compiled set of data. Split reflections could not be used for scaling but were included in the statistics. Their exclusion did not significantly improve the R factor.

boundaries of contiguous rotations were split between consecutive films, and such data were assimilated by adding the integrated intensities of the separate parts from each film¹⁰. Films were measured on a flat-bed scanner (J. F. W. Mallett, T. H. Gossling, A. R. Faruqi, and J.N.C., unpublished) which combines the positional accuracy of a mechanical stage¹¹ with the speed of a flying-spot densitometer¹². The data quality (Table 1) is poor by small protein standards because of the high proportion of weak reflections and the limited exposures, although these were already several hours per degree of oscillation on a rotating-anode X-ray tube. We accepted this lower standard, however, because of the expectation, which was later justified, that the 17-fold redundancy implicit in the data would compensate for this. Better data were obtained for the mercury derivative, for which larger crystals and longer exposures were used.

Phase determination

Phases were obtained by a combination¹³ of isomorphous replacement and non-crystallographic symmetry^{13,14}. The mercury positions found by Gilbert and Klug⁸ from the centrosymmetric zones were refined using 20,000 selected reflections. The refined positions were used both to locate the non-crystallographic 17-fold axis precisely and to calculate a single isomorphous map. The asymmetric unit was isolated, averaged 17-fold, and the averaged structure replaced in the unit cell. The averaged map was then used to compute a set of structure factors (F_{calc}). Treating the map

Table 2 Progress of the phase determinations starting from single (SIR) and double (DIR) isomorphous replacement

	SIR			DIR		
Cycle	0	1	2	0	1	2
R factor after averaging		0.38	0.35		0.39	0.31
Figure of merit	0.48	0.67	0.68	0.60	0.74	0.77
Mean phase change from last cycle		34.5°	3.4°		24.7°	6.5°

$R = \langle |F_{\text{obs}} - F_{\text{calc}}| \rangle / \langle F_{\text{obs}} \rangle$, where F_{calc} is the structure factor computed from the average map.

as the known part of a total structure, phase-probability distributions were computed from the F_{calc} using Sim's formula¹⁵. The sharpness of the distributions depends on the agreement between the measured amplitudes (F_{obs}) and the F_{calc} over the complete set of data. These distributions were combined multiplicatively with those obtained from isomorphous replacement to calculate new best phases and figures of merit. Attaching these to the F_{obs} , another map was calculated, enabling the cycle of averaging and phase-recombination to be repeated.

After only two or three cycles, the mean phase change per cycle was as little as 3° . Phases from the final cycle were used to locate the heavy atoms in the gold derivative, and ultimately the above procedure was repeated starting from double isomorphous phases. The course of each phase determination is summarised in Table 2. The final mean figure of merit was higher for the determination starting with the double isomorphous phases (0.78) than for that starting with the single (0.68). Nevertheless, the final maps in each case were of comparable quality, showing that the use of the non-crystallographic symmetry was of greater value than the second isomorphous derivative. The heavy-atom parameters for the two derivatives are given in Table 3. The hand was determined from the anomalous differences in the derivative data.

The electron density map and its interpretation

The map in Fig. 1 is the 17-fold averaged structure and is plotted on sections perpendicular to the 17-fold axis. The figure shows approximately $4\pi/17$ rad of the disk (two subunits wide) sliced into four thick composite sections, A1, A2, B1 and B2, each about half a layer deep. The two layers are denoted A and B, where A is closer to the crystal dyad, and each ring is divided into two parts, 1 and 2, to give the four sections (Fig. 2). Each layer contains two slewed rods of density (LS, RS) per subunit in the upper half and two radially oriented rods (LR, RR) underneath.

Table 3 Heavy atom parameters

	Mercury		Gold	
	Ring A	Ring B	Ring A	Ring B
Relative occupancy	58	58	23	14
Radius in Å	59.2	58.9	72.5	70.1
Height above dyad in Å	12.1	38.8	3.5	28.9
Azimuth (ϕ)	4.1°	8.0°	-0.6°	3.2°
Phasing power $\langle f_H \text{ calc} \rangle / \langle E \rangle$	2.78		1.10	

$f_H \text{ calc}$, Calculated heavy atom structure factor.

E , lack of closure error.

The 34 sites for each derivative were refined against the phases calculated by combining the probability distributions for the DIR phases (in which the weight of the gold contribution had been doubled) with those resulting from two cycles of averaging and recombination of the SIR phases. This procedure was necessary to prevent the mercury derivatives from completely dominating the DIR phase calculation and refinement. The Table gives the 17-fold averages of the parameters refined for individual atoms. None of the mercury positions differed by more than 0.3 Å from those corresponding to perfect symmetry.

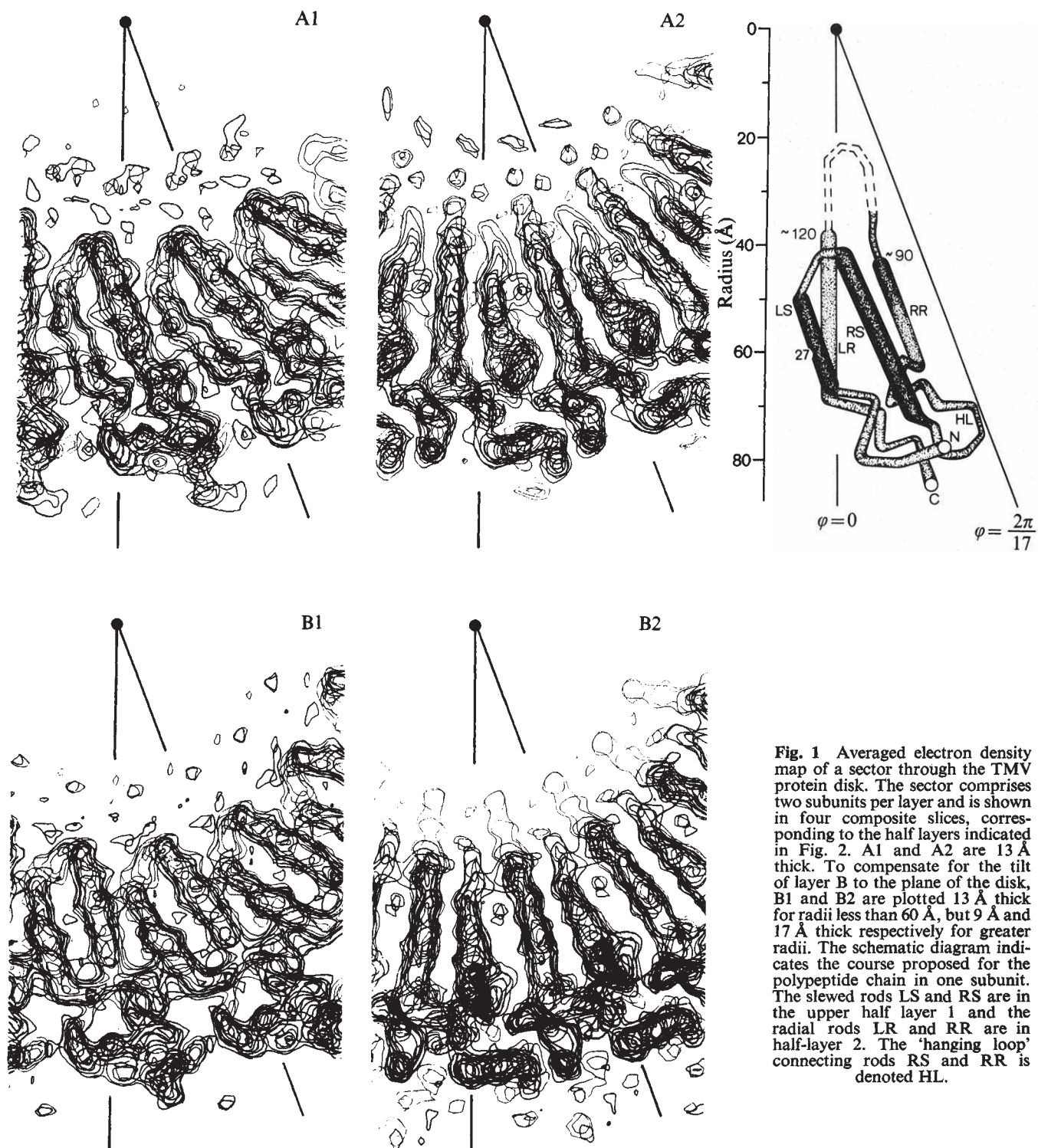


Fig. 1 Averaged electron density map of a sector through the TMV protein disk. The sector comprises two subunits per layer and is shown in four composite slices, corresponding to the half layers indicated in Fig. 2. A1 and A2 are 13 Å thick. To compensate for the tilt of layer B to the plane of the disk, B1 and B2 are plotted 13 Å thick for radii less than 60 Å, but 9 Å and 17 Å thick respectively for greater radii. The schematic diagram indicates the course proposed for the polypeptide chain in one subunit. The slewed rods LS and RS are in the upper half layer 1 and the radial rods LR and RR are in half-layer 2. The 'hanging loop' connecting rods RS and RR is denoted HL.

The structural similarity between the two crystallographically independent layers of subunits in the disk is indicative of the high quality of the phase determination. The map confirms earlier results that the disk is polar and has a pairing interaction between layers at high radius^{2,9}, as is clearly seen in side view (Fig. 2).

The precise relationship between the two layers is most easily found by comparing features common to each layer in cylindrical sections of the map (for example Fig. 3). The two are related by an azimuthal shift of about 4°, constant for all radii, whereas the axial separation of common features decreases linearly from 30 Å at low radius to 22.5 Å

at high radius, compared with a constant separation of 23 Å in the virus helix. At the present resolution, therefore, the interaction between layers does not involve any appreciable distortion of the subunits, but rather a bodily tilting by 10° of the subunits in one ring with respect to the other. The large gap between the two layers at a radius of 40 Å evidently allows the initial accommodation of the RNA, since the latter is at this position in the virus¹⁶.

The rods of density between radii 40 Å and 70 Å, are strongly suggestive of α -helices. They are approximately equispaced, 10 Å apart, within each layer so that there is little distinction between inter- and intramolecular contacts

in this region. The rods make angles of 10–20° with each other in a left-handed relationship, as is appropriate for right-handed α -helices. The two slewed rods are connected at 40 Å radius, showing that they belong to the same subunit. At high radius there is a 'hanging loop' connecting the right slewed rod (RS) to the right radial rod (RR) beneath it. The bottom of this loop is in close contact with the left radial rod (LR) of the neighbouring subunit. The simplicity of the arrangement of four dense rods extending from 45 Å to 65 Å contrasts sharply with the highly contorted density towards the outside of the molecule. Interpretation in this region is difficult but is helped by having two independent copies of the subunit, one in each layer. The subunit boundaries in the axial direction are particularly easy to locate because of the clear separation both between disks and within a disk. The chain tracing finally chosen is shown in Fig. 1 together with the electron density map. The subunit delineated is compact, tapering at low radius, broadening and bending azimuthally at high radius.

One disappointing feature of the map is the lack of density at radii below 40 Å; in the helical aggregate of TMV protein¹⁷ 20% of the density lies in this region. The radial rods in our map both peter out at 40 Å radius, suggesting that they are connected at lower radii but that this region is disordered in the disk. The absence of density in the map cannot be accounted for by chain cleavage, since sodium dodecyl sulphate (SDS) gels of old crystals show that virtually all the protein is of the correct size. A second possibility, that the 17-fold symmetry holds only at radii higher than 40 Å so that averaging the map smears out the true structure at lower radii, was discounted on inspection of the unaveraged map which shows no extra density at low radius.

A comparison of our map with that of the virus obtained in Heidelberg by Holmes *et al.*¹⁸ reveals reasonable agreement in the gross features. The connections between the helical regions in our protein subunit, however, do not agree with those derived from their lower resolution map. Both maps show four rods of density at intermediate radii, of which the lower two are more or less radial and the upper two are both slewed to the right. We identify our left and right radial rods with ribbons 3 and 2 respectively of the virus map, and our left- and right-slew rods with ribbons 1* (of the next subunit) and 4, but the connectivity is quite different. Starting from the N-terminus, our order is LS, RS, RR, LR, corresponding to 1*, 4, 2, 3 in the Heidelberg notation. The differences in interpretation arise because the clear connection between our slewed rods at 40 Å radius is missing in the virus map and because our map shows no connecting density between the radial rods at 70 Å radius in contrast to the strong loop between ribbons 2 and 3 in the virus map. The radial rods in our map pass continuously into the density at high radius, whereas in the virus map the latter forms an isolated 'island'.

Folding of the polypeptide chain

The most reliable information for correlating the course of the polypeptide chain (Fig. 1) with the sequence of 158 amino acid residues comes from the location of residues 1, 27, 115 and 139 labelled by heavy atoms in the virus^{18,19} and of residue 27 in the disk. There are also indirect data, reviewed by Durham and Butler²⁰, mainly concerning the accessibility of residues and regions of conserved sequence. The conservation of positively charged residues in all known strains and mutants at positions 41, 90, 92 and 113 suggests the proximity of these residues in the virus to the sugar-phosphate backbone of the RNA at 40 Å radius.

Arginine 41 can be assigned to the neighbourhood of the bend at 40 Å connecting the LS and RS rods, since cysteine 27 is located by the mercury atom at a radius of

60 Å close to the LS rod (Fig. 1), while the N-terminus is at high radius¹⁹. The other conserved arginines are therefore probably situated on the radial rods near the RNA radius. This is consistent with putting the stretch of sequence 90–120 at still lower radii, for which there is indirect evidence²⁰, and thus assigning this region to the invisible chain connecting the two radial rods. The interaction of RNA with protein would then involve arginine 41 of the subunit below the RNA and arginines 90, 92 and 113 of the subunit above it.

The availability of the C-terminal residue to carboxypeptidase digestion in the intact virus²¹ suggests that this is on the outside, at high radius. Residue 139 can be located approximately between the outer continuations of the radial rods by relating the coordinates of the dimercure acetic acid and methylmercury sites in the virus¹⁸ to the methylmercury site in the disk. We choose the left-hand rod for 139, and thus the right-hand rod for 90, as this gives a more plausible connection of the remaining density than does the reverse. The left-hand radial rod is connected to the C-terminus at high radius through density in the lower half of the subunit.

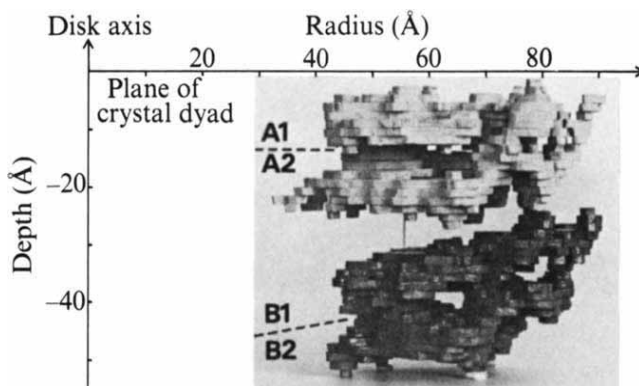


Fig. 2 Side view of a balsawood model of two subunits in each layer of the protein disk. The layers interact closely only at high radius, subunits in ring B being tilted away from subunits in ring A at low radius.

Our interpretation of the map allows the rods to be in the α -helical configuration over most of their length, and two stretches of sequence rich in hydrophobic residues (74–87 and 119–133) are assigned within the radial rods RR and LR respectively. Parts of the RS and RR rods, however, may be more extended than a regular α -helix so that the combined length of the two rods and the 'hanging loop' connecting them can be spanned by residues 41–90. The chain tracing proposed here is not the only one conceivable, but accords best with the electron density map. The most uncertain feature concerns a four-way contact at 70 Å radius involving the RS and RR rods and the connecting 'hanging loop'. The direction of the sequence through this loop may be the reverse of that proposed here.

Subunit packing

The subunits within a layer are tightly packed, the principal contacts being those associated with the α -helical packing in the region of radii 40–70 Å. There are only two main contacts between layers and these can be seen in Fig. 2 at radii 65 and 80 Å. In the virus, however, lateral and axial contacts between subunits seem to be equally close.

The surface lattices of the disk structure and the virus helix are compared in Fig. 3. The 16-fold helix family in

the virus has a one-third subunit displacement to the left²² between turns, whereas the two rings of the disk have a relative azimuthal displacement of about one-fifth subunit to the right. Thus the geometry of axial contacts between the two rings of the disk is quite different from that between successive turns of the virus helix. Figure 3a shows a cylindrical section of the electron density map of the

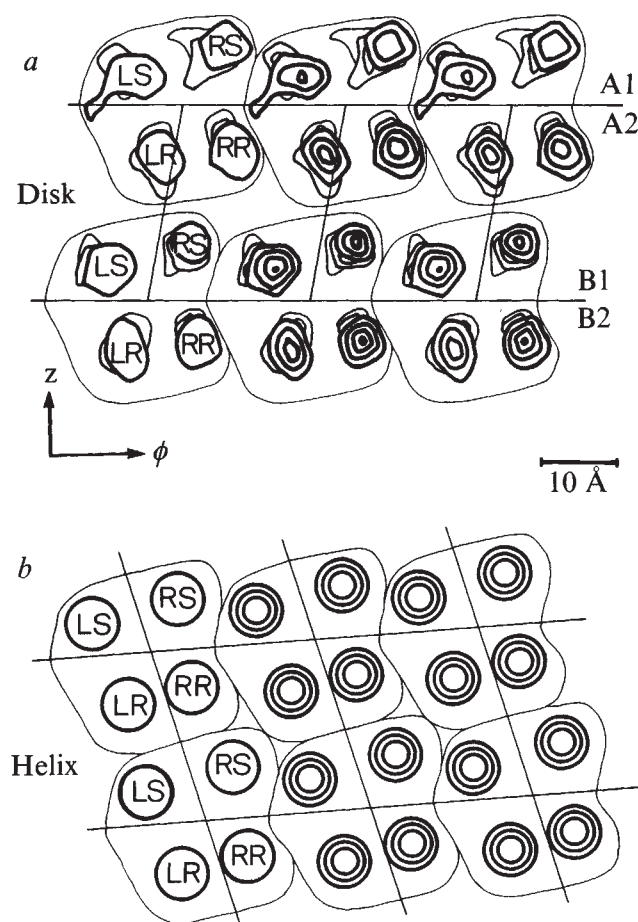


Fig. 3 Packing of subunits and α -helical rods in the protein disk and the virus. *a*, Superposed cylindrical sections of the electron density map of the disk at radii 57, 58.5 and 60 Å. The subunit outline is shown. *b*, Schematic diagram of the same subunits arranged in the surface lattice of the virus helix.

disk at a radius of about 58 Å illustrating the packing of the α -helical rods. The rods are well packed laterally within a layer of the disk, but there is only one close contact between layers. If, however, we generate the packing in the virus in this region by applying the appropriate displacement to one layer such that the surface net of the disk is converted to that of the virus (Fig. 3b), the layers now fit snugly. Further contacts are made giving a continuous two-dimensional array of close-packed rods.

Implications for virus assembly

The disk has a primary function in both the initiation of the virus structure and its subsequent elongation^{5,6} and the structure we have found suggests how this might occur. The disk is not just a simple piece of virus-like structure without the RNA, but it is an intermediate assembly of protein molecules designed for incorporation of the RNA before subsequent transformation to the final helical

arrangement. The contacts between layers of the disk are few, and at the RNA radius the subunits of the disk are about 7 Å further apart axially than they are in the virus. The two layers of the disk thus have the appearance (Fig. 2) of an open pair of 'jaws', awaiting the incorporation of the RNA. We would expect initiation of assembly to involve the reaction of a single disk with the RNA, as has been found to be the case (ref. 6, p. 343).

As the RNA binds to the disk at 40 Å radius, the inner disordered region presumably adopts the virus configuration, the bonds between layers at higher radius being broken as the new bonds characteristic of the virus configuration (compare with Fig. 3) are formed. The disk would presumably dislocate to a helical segment at some point in this process, the RNA beginning its second turn. Another disk could then attach to start the regime of rod elongation. There is no need to postulate distinct mechanisms for initiation and elongation; the flexibility of the chain at low radius would facilitate intercalation of the RNA between the protein subunits, thus resolving the paradox of elongation from a two-layered structure—the disk²².

The disorder of the protein in the disk at low radius may be symptomatic of a somewhat nonspecific chain configuration at low radii in the virus, where a unique protein sequence has to accommodate a variety of RNA base triplets in different subunits. This disorder in the disk suggests that the protein does not carry three preformed sites, of the lock-and-key kind, to house the RNA, but rather that the sites are created during the interaction between them. Nevertheless, there must be sufficient stereospecificity in the disk to recognise the special initiation sequence of the RNA, although not all successive bases of this sequence need be involved.

Further details of the interactions between subunits and between protein and RNA, together with confirmation of the folding we propose for the protein subunit must await a 2.8 Å resolution map, the data collection for which is now in progress.

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letters to nature

Pulsar atmospheric current loops

THE basic difficulty with the theories of pulsar radiation is that there is as yet no self-consistent model of the large scale structure of the plasma atmosphere of the pulsar^{1, 2}. The lack of such a model is perhaps most strikingly exemplified by the fact that there is no consistent explanation of how an aligned rotating magnetised neutron star can maintain a net zero current from its surface—that is, how either a finite current loop in its atmosphere, or an 'infinite' loop to the nebula, is established consistent with the space charge and the electromagnetic fields^{3, 4}. This letter will be limited to a discussion of how current loops are established when the rotational and magnetic axes are aligned.

To avoid the extreme conditions which may exist in real pulsars, we will use a gedanken device to tackle the problem. The rotational state of the star will be obtained by beginning with a magnetised star in a vacuum, and adiabatically increasing its angular velocity $\Omega(t)$. In this way the various physical complications can be introduced in a systematic manner—ordered according to the value of Ω . Our concern is essentially limited to the lowest order influence of Ω , which requires a resolution of the current loop problem.

The rotation of a conducting star about its dipole magnetic field axis, induces an electric field which must satisfy the boundary condition

$$E_\theta(r = R, \theta) = - (Q/2R^2) \sin 2\theta \quad (1)$$

where $Q = (\Omega \cdot \mathbf{B}_0)R^3/c$, and $\mathbf{B}_0$ is the polar magnetic field strength. Goldreich and Julian³ recognised that for real pulsars this yields very large values of $\mathbf{E} \cdot \mathbf{B}$, causing large currents from the star, hence invalidating the vacuum assumption. They then made the assumption that $\mathbf{E} \cdot \mathbf{B} \approx 0$ which, together with equation (1), leads to a corotational atmosphere and their paradoxical result concerning 'proton' currents passing through

negatively charged regions⁴. They also introduced the assumption of a group of open magnetic surfaces, along which current flows to the nebula. These basic assumptions, which have been widely accepted in the literature, are totally different from the model proposed here.

If we take Ω to be small (hence small Q), the Goldreich and Julian vacuum field would initially draw only electrons from the star, primarily from the polar regions. This would give rise to a stellar charge $q_s = \epsilon Q$. To the degree that the electrons move to large distances, thus producing little space charge, the electrostatic potential which satisfies equation (1) is

$$\phi = \frac{Q}{r} \left[\epsilon - \frac{1}{6} \left(\frac{R}{r} \right)^2 (3 \cos^2 \theta - 1) \right] \quad (2)$$

and

$$\mathbf{E} \cdot \mathbf{B} = \frac{QB_0}{R} \left[\epsilon - \left(\frac{R}{r} \right)^2 \cos^2 \theta \right] \left(\frac{R}{r} \right)^5 \cos \theta \quad (3)$$

The effect of the stellar charge is to slow down and eventually stop any net electron escape from the star. This will occur approximately when the polar potential equals the nebular potential at some nebular distance ($r = R_N$). For $R_N \approx \infty$, $\phi(R, \theta = 0) = \phi(\infty, \theta = 0)$ if $\epsilon = 1/3$. This net stellar charge, $q_s = Q/3$, does not stop electron emission from the polar region, as can be seen from equation (3). The problem therefore remains to determine the flow of these electrons which can no longer escape into the nebula. The details of this plasma flow is not particularly simple, and will be discussed in a later publication. To give a qualitative (and simplistic) picture, it is of some help to introduce regions in which the plasma flow is dominated by either the electric or magnetic field:

magnetic flow region (MFR): $|\mathbf{E} \times \mathbf{B}| > E^2$

electric flow region (EFR): $|\mathbf{E} \times \mathbf{B}| > B^2 \quad (4)$

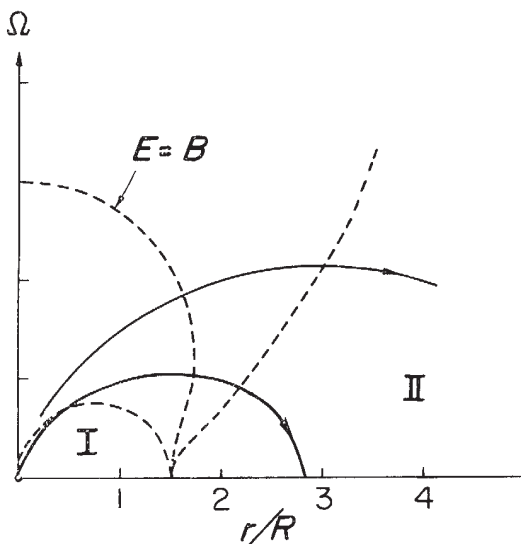
In these regions the average acceleration of the particles is along $\mathbf{B}$ (in the MFR) or $\mathbf{E}$ (in the EFR), and in an intermediate direction in the region near $\mathbf{E} = \mathbf{B}$. These regions are illustrated in Fig. 1 for the dipole $\mathbf{B}$ field, and $\mathbf{E}$ obtained from equation (2), with $\epsilon = 1/3$. The (toroidal drift velocity/c) $\equiv \beta$, given in ref. 5 by $\beta/(1 + \beta^2) = (\mathbf{E} \times \mathbf{B})/(E^2 + B^2)$, is not corotational and generally $\beta \neq 1$ any place on a field line. The essential point to note is, however, the flow across magnetic surfaces in the EFR (a point which apparently has not been noted in the force-free approximation).

If electrons start from rest at (R, θ_0) and move out along the dipole field lines $r = R(\sin \theta / \sin \theta_0)^2$ (Fig. 1), where the field line is labelled by its stellar surface angle θ_0 , they would be energetically able only to reach a point $(r^*(\theta_0), \theta^*(\theta_0))$ given by

$$\phi(r^*, \theta^*) \equiv \phi(R(\sin \theta^* / \sin \theta_0)^2, \theta^*) = \phi(R, \theta_0) \quad (5)$$

In this case, once arriving at $\theta^*(\theta_0)$ they would be reflected and return to the star. If, however, $\theta^*(\theta_0)$ falls in the EFR, the electron will accelerate across magnetic surfaces to surfaces with larger values of θ_0 (lower electrostatic energy). [Note that this fixation on the EFR is a dynamic simplification, which neglects some 'shorting' across magnetic surfaces which begins when $E > B$ (Fig. 1). The present concern is conceptual rather than

Fig. 1 Plasma flow regions: acceleration along $\mathbf{B}$ for the MFR (region I), along $\mathbf{E}$ for the EFR (region II), or in intermediate directions ($E \approx B$). The star is at the origin. The solid lines follow dipole lines: $r = R(\sin \theta / \sin \theta_0)^2$.



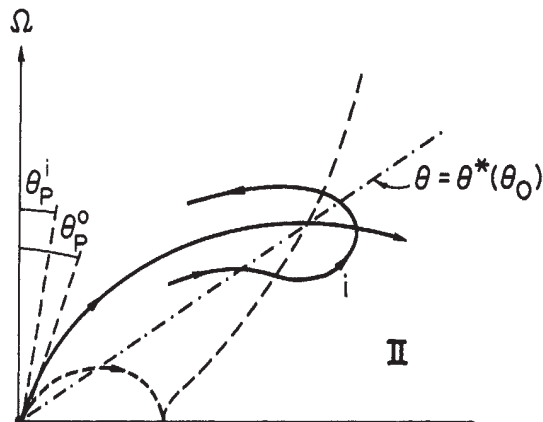


Fig. 2 Schematic of the primary electron current loop which crosses magnetic surfaces in the EFR. Inner and outer polar angles (θ_p^i , θ_p^o) at the stellar surface are defined by the indicated magnetic surfaces.

analytic.] This, then, distinguishes three groups of magnetic surfaces: (a) those such that $(r^*(\theta_0), \theta^*(\theta_0))$ lies in the EFR; (b) those surfaces which enter the EFR, but for which (r^*, θ^*) is not in EFR; (c) those surfaces which do not enter the EFR. The stellar surface angle which separates the group (a-b) and (b-c) define an inner polar angle (θ_p^i) and an outer polar angle (θ_p^o) respectively. These features are illustrated in Fig. 2.

This model predicts that the electrons which flow out along the magnetic surface $\theta_0 < \theta_p^i$ will enter a region (for example the EFR) where they cross magnetic surfaces to surfaces with larger θ_0 . In the process they will leave the electric-dominated region and then follow their new magnetic surface back to the star—forming the required current loop. The return flow of electrons will be largely restricted to the stellar annulus $\theta_p^o > \theta_0 > \theta_p^i$, the larger angle corresponding to the outermost surface to enter the EFR. An examination of the potential energy obtained from equation (2) shows that this flow pattern is consistent with the fields. This flow is schematically illustrated in Fig. 2. It will be noted that this current loop does not extend to the nebula, and does not represent any particle loss from the star (in contrast with large particle losses assumed in many pulsar models).

The present results are only valid to lowest order in Ω . The use, however, of an adiabatically increasing $\Omega(r)$ not only solves the immediate problem, but also gives a number of interesting side-features. The simple current diagram in Fig. 2 in reality consists of a 'plume' or 'fountain' particle flow formation, which cannot be described by simple hydrodynamic equations, and is quite likely unstable. Moreover, equation (2) exhibits a saddle-point potential energy minimum above the poles, which can naturally account for a stationary plasma atmosphere. The gedanken approach shows that this atmosphere, while requiring more stellar electrons, will not change the net star-plus-atmospheric charge, and thus the long range Coulomb force on which the present results rest. These features of a more complete pulsar atmospheric model will be published in the near future.

Finally it should be noted that the details of our results will be significantly changed if the rotational and magnetic axes are not aligned. This is clear from the spatial scale shown in Fig. 1. The analysis of the non-aligned case will be presented in a separate paper.

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An interstellar H₂ indicator in direction of the Crab Nebula

MOLECULAR hydrogen is very stable at low temperatures, and is therefore likely to be found in large quantities within interstellar dark clouds which are not penetrated by ultraviolet radiation (which destroys molecular bonds).

A case for a high concentration of H₂ in the interstellar medium can be made on the observed distribution of the galactic γ -ray emission^{1,2}, but available information is still rather scanty as direct evidence of its presence comes only from regions which are very close to the Earth. For regions further out than 1 kpc, the experimental evidence is only indirect—obtained from CO microwave emission.

We have studied the shape of the low energy spectrum of galactic X-ray sources detectable on Earth. In the energy range 0.1–1 keV the continuous absorption by H₂ is intense. It adds to that produced by other components of the interstellar medium and affects the depth of the absorption edges of elements such as oxygen, neon and others. The oxygen feature at 0.532 keV is very prominent and allows a sensitive probe of the H₂ density between the X-ray source and the observer. We therefore calculated how such features in the low energy X-ray spectrum of the Crab Nebula would be affected by the interstellar abundance of molecular hydrogen.

H₂ between us and the Crab would not affect the propagation of the 21 cm radiation³, but we find that it would absorb X-rays. This accounts for a factor of 2 in the difference between X- and radio-estimations of the column density of the interstellar medium in this direction. Indeed, the higher X-ray column density cannot be explained by interstellar grains or by any anomalous abundance of the usual components of interstellar matter, except on very specific models which lack any experimental support⁴⁻⁶.

We made the following assumptions in our calculation: first the X-ray energy spectrum of the Crab Nebula in absence of absorption follows a power law

$$N(E)dE = KE^{-\gamma}dE$$

of constant spectral index γ .

Extrapolation to low energy of the spectrum measured above a few keV, where absorption is negligible, gives $\gamma = 2.0$

Fig. 1 The X-ray energy spectrum of the Crab Nebula modified by interstellar absorption as seen with infinite resolution, $\Delta E = 0$, (a), and finite resolution, $\Delta E = 150$ eV, (b), for different values of H₂ abundance. — — —, $R = 0$; ———, $R = 0.5$.

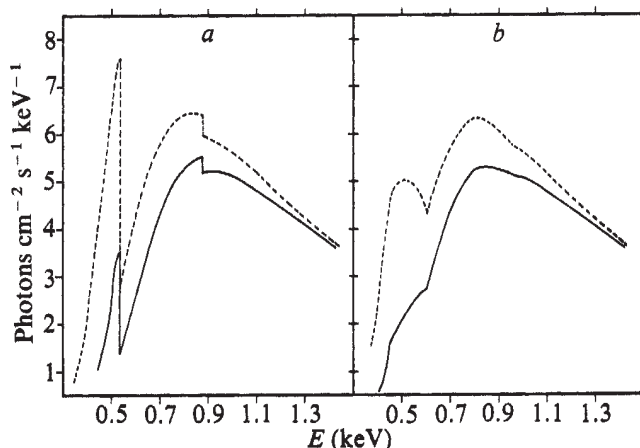


Table 1 Depth D of the oxygen absorption edge as a function of the H_2 abundance R

R	$D(R)/D(0)$
0	1
5×10^{-3}	0.996
10^{-2}	0.980
3×10^{-2}	0.948
10^{-1}	0.856
3×10^{-1}	0.628
5×10^{-1}	0.460
7×10^{-1}	0.337

(refs 4, 5, 7). Second, the column density of atomic hydrogen is 2.0×10^{21} atoms cm^{-2} compatible with radio measurements at 21 cm^8 . Third, except for molecular hydrogen, the composition of the interstellar medium is standard. The absorption cross sections and abundances computed by Brown and Gould⁹ have been adopted, and fourth, in the energy range of interest the absorption cross section of H_2 has the form

$$\sigma_{H_2}(E) = 1.84 \times 10^{-22} E^{-3.3} \text{ cm}^2$$

Results of the calculations are presented in Fig. 1 and Table 1. Figure 1a shows the expected Crab Nebula low energy X-ray spectrum assuming $R = 0.0$ or $R = 0.5$ (where $R = 2N_{H_2}/N_H$, N_{H_2} and N_H being respectively the densities of molecular and atomic hydrogen) and infinite resolution, ($\Delta E = 0$). When H_2 is present in the interstellar medium the depth of the absorption edges decreases. Table 1 gives, for various H_2 abundances, the depth of the oxygen feature divided by the depth of the feature when $R = 0$. Figure 1b gives the same spectra as Fig. 1a calculated under the hypothesis that the best energy resolution now available at 0.5 keV is used, $\Delta E = 150$ eV. The neon edge at 0.874 keV practically disappears. The oxygen absorption edge, indicated by a bump in the smooth trend of the spectrum, is now the only feature which stays

Table 2 Height H of the oxygen bump in units of $h\sigma$, (see text), as a function of the H_2 abundance R

R	$H/h\sigma \text{ (cm}^2 \text{ s)}^{1/2}$
0	1.47
10^{-2}	1.46
3×10^{-2}	1.44
10^{-1}	1.36
3×10^{-1}	1.17
5×10^{-1}	1
6×10^{-1}	0.92

visible. To detect it in an experiment carried out with a detector of area A , efficiency η and energy resolution ΔE , the Crab must be observed for such a long time t that the bump stands up by at least 3σ above the continuum.

Table 2 gives the height of the oxygen bump in units of $h\sigma$ where

$$h = (g(\Delta E)\eta A t)^{1/2}$$

and $g(\Delta E)$ takes into account the energy resolution of the detector. $g(\Delta E) \leq 1$ and tends to 1 when $\Delta E \rightarrow 0$; a rough estimate based on comparison between Fig. 1a and b gives $g(150 \text{ eV}) \simeq 0.3$. In the present experimental situation ($A \geq 100 \text{ cm}^2$, $t > 60 \text{ s}$, $\eta \simeq 0.2$) values of $h > 10 \text{ (cm}^2 \text{ s)}^{1/2}$ are common and $h \simeq 1,000$ possible (on satellite experiments). The oxygen feature is therefore a sensitive indicator of the molecular hydrogen abundance in direction of the Crab Nebula.

At present, data on the soft X-ray flux from the direction of the Crab Nebula are available only at energies near and above 1 keV, where the absorption effects are completely within the present experimental uncertainty of the measured flux. We cannot therefore carry out comparisons. In the near future,

however, observations particularly aimed at observing the oxygen feature, (Griffiths, personal communication), will extend the available information towards lower energies and allow significant comparisons between theory and experiment.

Some comments are necessary about the H_2 absorption cross section. Between 0.1 and 1 keV there are estimates of σ_{H_2} which differ by a factor as high as 4.5 (ref. 10) from the one we used and give rise to an oxygen absorption edge higher by up to a factor $\simeq 2$ at $R = 0.5$. In the absence of firm theoretical and experimental indications, we decided to use the most widely adopted cross section value in order to obtain results easily comparable with other calculations.

In spite of such an uncertainty, comparisons between calculated and measured spectra of the Crab Nebula X-ray flux are still worthwhile.

Application of this method to other X-ray sources, for which observations at sufficiently low energy already exist (see for instance ref. 11), can be made provided that first, the source distance or the hydrogen column density in the source direction is known and, second, reasonable assumptions on the source X-ray spectrum in absence of absorption are possible.

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Transient short time periodicities in the optical emission from Cyg X-1

TEMPORAL structure down to the millisecond time scale has been observed in the X-ray emission of Cyg X-1 (ref. 1). This has been interpreted as evidence of instabilities in the accretion disk surrounding a black hole²⁻⁴, lasting for approximately the orbital period of the infalling matter. We report here observations of modulated optical emission from this source with a period of ~ 83 ms. The modulated emission was detected in two sets of observations as transient events lasting ~ 10 min. This is the first evidence of strong emission at optical wavelengths from the X-ray source in the Cyg X-1-HDE226868 system.

The short period variations in optical emission were investigated with a photometric arrangement on the 91-cm telescope at the Catania Astrophysical Observatory. Single photon pulses and 1 kHz reference signal were recorded on magnetic tape, and the counting rate for 1-ms intervals subsequently transferred to digital tape. Computer analysis included correction for variable atmospheric absorption, Fourier analysis with the Cooley-Tuckey (FFT) algorithm and count rate folding. The time base accuracy is 1 part in 10^8 , achieved by daily checking against the BIH international time scale using a 5-MHz radio link.

Systematic observations of both comparison stars and Cyg X-1 (when quiet) confirms that scintillation noise cannot

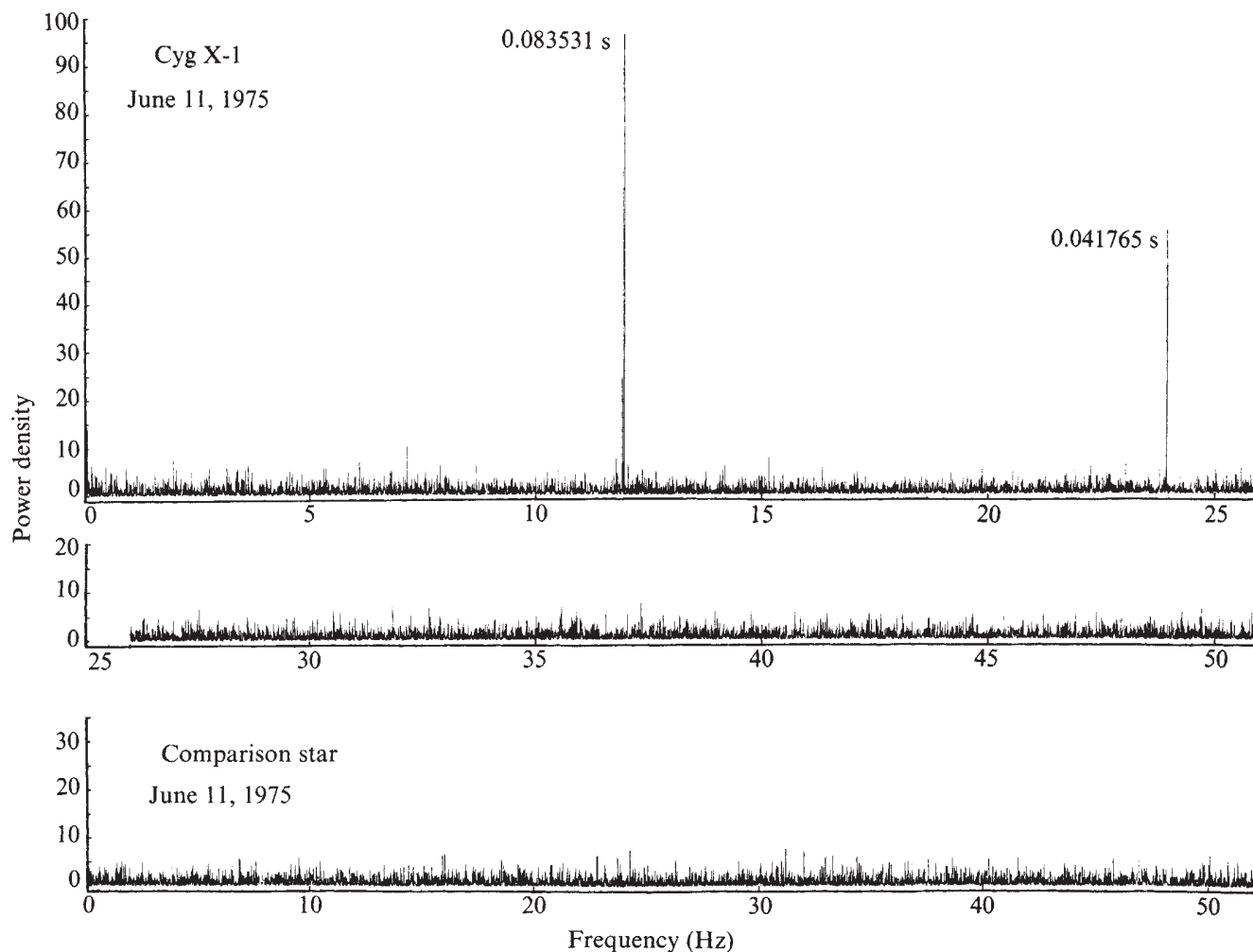


Fig. 1 Power density spectrum of the event of June 11.06111 and of the comparison star.

simulate a statistically significant modulation above a few Hz. The statistical distribution of the fluctuations in the Fourier spectrum is quite consistent with that expected from counting statistics, and we therefore deduce a sensitivity for our survey of 0.1% pulsed fraction with 99% statistical confidence in the frequency range 5–500 Hz.

Routine observations of Cyg X-1 were carried out in November 1973 and September 1974 with negative results. Observations were repeated in June 1975 after the observation of a new flare by the X-ray equipment on the ANS satellite⁵. Analysis of the data has revealed that a strong pulsed component appeared at 01.28 UT, June 11. The pulsation with a period of 83.531 ± 0.008 ms lasted for ~ 10 min. After this event no significant power was detected in the Fourier spectrum of Cyg X-1, nor in the check performed on the comparison star soon afterwards (Fig. 1).

The light curve of the pulsation, obtained by folding the counting rate module 83.531 ms, shows a very regular structure. The periodicity detected by Fourier analysis is caused by falls of intensity with a duty cycle near 25% (Fig. 2) naively suggesting an eclipsing phenomenon.

Figure 3 shows the time evolution of this event in steps of 30.72 s from the beginning of the tape to the merging of the pulsation in the background fluctuations. In this plot we have a clear picture of the settling of the pulsations. In fact, we observe that the modulated component is superimposed on the steady-state luminosity of the source. This brightening can be

also observed in a plot of the counting rate integrated for 1 s. On this plot the modulation growth has a rise time of ~ 3 s. It should be noted that the modulation period was not strictly

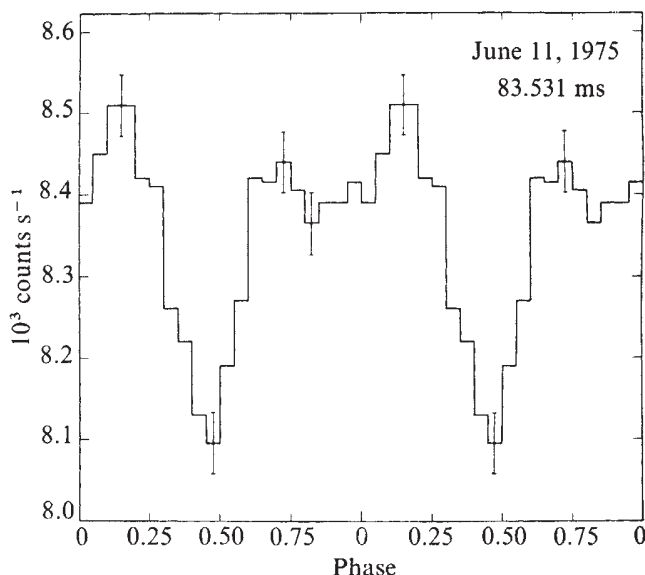


Fig. 2 Light curve of the modulated emission, obtained by folding ~ 100 s of data.

Table 1 Data from flare events on Cyg X-1

Time	Period P (ms)	$\Delta P/P$	Filter	Duration (min)	$\Delta L/L$
June 11.06111 UT	83.531 ± 0.008	10^{-4}	Grey ($\times 10$)	10	3.5 per cent
July 18.00410 UT	83.714 ± 0.008	2×10^{-4}	R	> 10	2.5 per cent
July 18.03674 UT*	83.592 ± 0.008	2×10^{-4}	B	> 6	3.7 per cent
July 18.04854 UT*	83.592 ± 0.008	2×10^{-4}	Grey ($\times 10$)	> 8.5	4.2 per cent

*Probably related to the same event.

constant during the event, as shown by a significant variation in the phase of the minima. We estimate that during the event the period fluctuated by ~ 1 part in 10^4 .

A similar activity in the source was observed one month later. On July 18 several events occurred in the space of ≈ 1 h. The data are given in Table 1. The light curves of the events in

because of the large luminosity of the spots which could come only from a non-thermal process.

Alternatively, if the phenomenon is associated with a rotating body, one cannot explain the variations of the period observed without assuming a triple star. In this case the period variability implies an orbital velocity $> 300 \text{ km s}^{-1}$ for the rotating body.

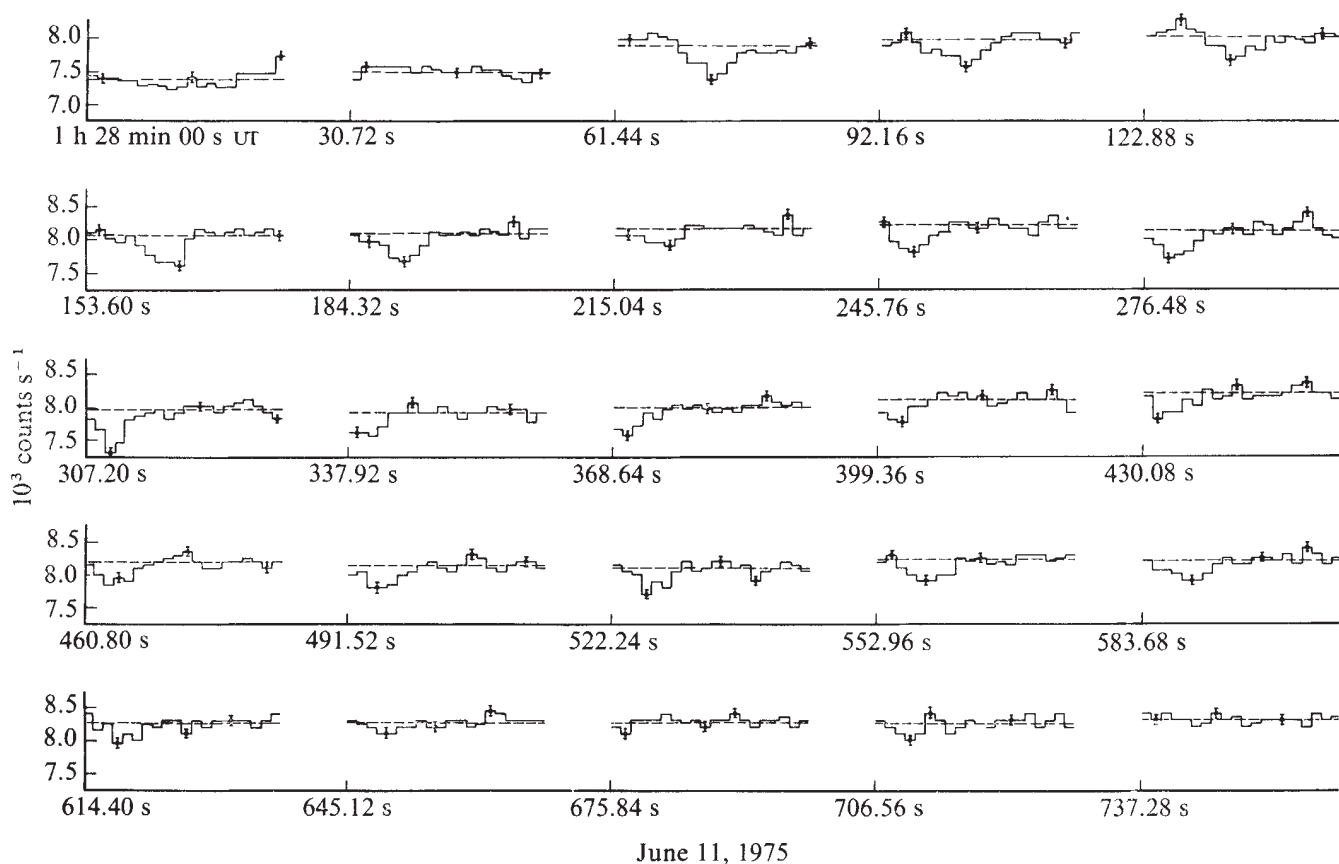


Fig. 3 Evolution of the June event. Each plot corresponds to the folding of ~ 30 s of data.

July are quite similar to those of June except that the duty cycle of the drop in intensity is here closer to 50%. Some ambiguity can arise in the time separation of the events. We assumed that the period given by Fourier analysis (the mean period) identifies each individual event. We stress that differences in period are quite noticeable, although small (~ 1 part in 10^3). The luminosity of the modulated emission can be estimated assuming that the bulk of the optical luminosity comes from the supergiant blue star HDE226868, and that this has the absolute visual magnitude of -6.5 , corresponding to its spectral type. This gives a surprisingly high luminosity for modulated emission $\sim 10^{35}$ – $10^{36} \text{ erg s}^{-1}$.

Very qualitatively, such a periodicity in the optical emission could fit the popular accretion disk models for Cyg X-1. We may assume that this optical emission is generated by instabilities in 'spots' in the disk. Our data would then indicate that such instabilities arise in a rather limited region at a distance of a few hundred kilometres from the black hole. Difficulties arise

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Entropy production by black holes

HAWKING has shown¹ that black holes, treated quantum mechanically, emit black body radiation of temperature $T = 10^{-7} (M_{\odot}/M) \text{ K}$, and hence evaporate—if isolated—on a time scale² of $\sim 10^{66} (M/M_{\odot})^3 \text{ yr}$. Energy arguments^{1,3,4} suggest that a black hole can be assigned the entropy

$$S_{\text{bh}} = 4\pi GM^2 k / \hbar c \sim 10^{77} k \sim 2 \times 10^{18} S_{\text{H}}(M/M_{\odot})$$

where $S_{\text{H}} \sim 40(M/M_{\text{p}})k$ is the entropy of a hydrogen cloud from which the black hole is assumed to have formed, M_{p} is the mass of the proton, and k is Boltzmann's constant. This implies that when a hydrogen cloud of mass $1M_{\odot}$ collapses into a black hole, the entropy of the (uncollapsed) galaxy [$\sim 10^{12} S_{\text{H}}(M_{\odot})$ and more] would increase by a factor $\sim 10^8$. In other words: something hardly observable, namely the collapse of one star, would increase the entropy of our cosmic neighbourhood by a large factor. We endeavour to resolve this apparent puzzle.

To begin with, it must be remembered that in 'normal' conditions, matter has an entropy particle $s \sim 1$, ($s = SN^{-1}k^{-1}$) where N = particle number; s varies between $\lesssim 1$ (for example for electrons at temperatures T below the Fermi temperature T_{F}) and $\lesssim 90$ (for dispersed hydrogen of critical cosmological density $a \sim 10^8 \text{ K}$); $s \sim 4$ for an extreme relativistic ideal gas. When a star forms by contraction of a cloud, s ($\sim -\ln(n\lambda^3) + 5/2$) decreases from $s_{\text{cloud}} \sim 40$ to $s_{\text{star}} \sim 10$ (where n = particle number density, $\lambda = h/(2\pi mkT)^{1/2}$ is the thermal de Broglie wavelength), but the second law of thermodynamics is not violated because the initial entropy has been converted into thermal radiation. If the star continues to shrink, its entropy

decreases still further, and again the excess entropy goes into thermal radiation. Following the matter through, we find a black hole of $1M_{\odot}$ to have a rather small relative entropy at formation smaller than that of a neutron star. On the other hand, an isolated black hole evaporates within $t_{\text{evap}} = 10^{66} (M/M_{\odot})^3 \text{ yr}$ and thereby emits black body radiation of temperature T . For complete evaporation, the vacuum must be large enough to take this black-hole energy. In filling this volume, the radiation randomises and acquires precisely the entropy given above; which is $10^{19} (M/M_{\odot})^2$ times larger than that at formation.

This suggests the following resolution of our puzzle: a black hole forms on the collapse time scale $t_{\text{coll}} = GM/c^3 = 10^{-5} (M/M_{\odot}) \text{ s}$, with an entropy at birth smaller than that of its building blocks. Thereafter, if left alone it irreversibly evaporates on the (huge) time scale t_{evap} , with a (small) entropy production rate $S \sim \dot{S}_{\text{bh}}/t_{\text{evap}}$. This implies that the entropy of the Galaxy is hardly affected by black hole formation. The so-called entropy of a black hole is the entropy it would produce during its irreversible evaporation.

Our suggested resolution can be made more explicit by noting that the entropy of an arbitrary thermodynamic system is defined as a hypersurface integral over the entropy four-current density s^a ,

$$k^{-1}S[\Sigma] = \int_{\Sigma} s^a dx_a^*$$

with s^a a future time-like vector inside the substratum (third law), $s^a = 0$ in vacuum, and $s^a \geq 0$ throughout (second law). In flat space-time, Σ is to be the hyperplane normal to the observer. In an asymptotically flat space-time, Σ will be chosen tangential to a distant observer's normal hyperplane; but in general there is a continuous arbitrariness in the choice of Σ in non-static domains. Such an arbitrariness implies that there will be a minimum and a maximum entropy for each space-time point x , namely the above functional S for the 'earliest' and the 'latest' Σ through x . $S_{\text{min}}(x)$ and $S_{\text{max}}(x)$ are equal for $s^a = 0$ in the enclosed domain, that is, if all thermodynamic processes taking place are reversible, and almost equal for slow processes. An extreme situation occurs when a black hole forms, because then Σ should avoid some ill-understood space-time domain inside the hole: at certain (distant) observer times, Σ must cut through the black hole interior which cannot be observed in the classical (non-evaporation) limit. But the Cauchy development makes unique predictions (in principle) which can be used to calculate $S[\Sigma]$. And a glance at Fig. 1 shows how a distant observer notices the evaporation of an isolated black hole. In this two-dimensional conformal space-time diagram, light rays propagate at 45° ; angular degrees of freedom are suppressed; the collapsing star is represented by some of its (time-like) worldlines including the worldline of its centre (with a classically singular portion marked '?'). Flashes of radiation are emitted during the collapse and at the end of evaporation (when $T(M)$ diverges, and a singularity forms). The entropies in the consecutive space sections $\Sigma_1, \Sigma_2, \Sigma_3$ are of order $S_{\text{H}}, \alpha S_{\text{bh}}, S_{\text{bh}}$ respectively, where α is smaller than, but of the order of, unity.

These statements are at variance with those of Hawking⁵ or earlier authors⁶ who assign a time-independent value S_{bh} to the entropy contained inside the event horizon of a black hole, and which I consider untenable for the reasons given above. In particular, S_{bh} is $10^{19} (M/M_{\odot})^2$ times larger than the entropy determined by local observers inside the black hole! And infalling observers cannot be discarded as 'unphysical': if our Galaxy contracted within its Schwarzschild volume, we would have another month to live under almost present-day conditions—not at almost infinite entropy density—for which period some of us may like to have predictions.

If we accept this, black holes of the same mass, spin, and charge can have different entropies, depending on their ways of

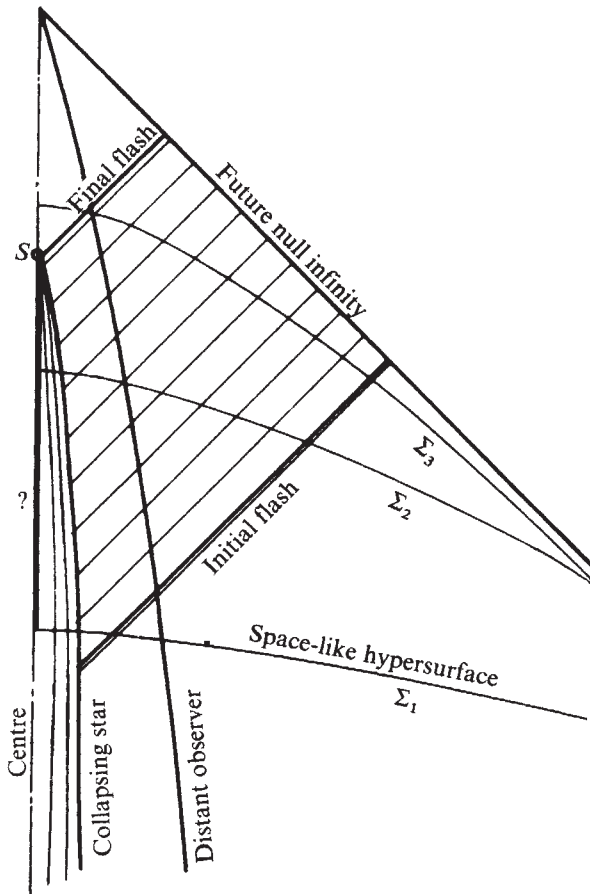


Fig. 1 Conformally finite radial space-time section through an isolated collapsing star (Penrose diagram).

formation. The highest entropy would be obtained for a black hole formed from the coldest possible radiation—radiation at its evaporation temperature. In this case, and only in this case, would evaporation be an isentropic process, S_{bh} being (already) the entropy at formation. Such black holes can exist in thermal equilibrium with their surroundings, but they cannot form⁵ spontaneously from radiation unless their mass is below $10^{-6}g$, (corresponding to temperatures above $10^{32}K$); they have to be synthesised from lower entropy ones.

What do we know about the entropy in the Universe? In our cosmic neighbourhood, the comoving entropy is nearly constant and given by that of the 3 K background radiation. Present-day matter contributes⁷, at most, a fraction 3×10^{-7} , (whose slow increase makes life possible). The comoving entropy would be maximal if all matter were converted into heat of uniform temperature; processing through black holes could in principle achieve this as local conservation laws (such as baryon number conservation) are thereby transcended. If this were achieved at time t ($\geq t_0$, the present time), cosmic entropy would be raised by a factor $\lesssim 10^8(t/t_0)^{1/2}$. In particular, present-day cosmic entropy is $\geq 0.1\%$ maximal.

This consideration arose from a conversation with H. Buchdahl, and was promoted by discussions with A. King and J. P. Lasota.

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Possible implications of the Rubin-Ford effect

AN anomaly in the distribution of radial velocities was reported for Sc 1 galaxies by Rubin *et al.*¹. They found different mean velocities in two nearby hemispherical regions (I and II, see Fig. 1), although the mean apparent magnitude was found to be the same for both sets.

One interpretation they advanced was that the Hubble constant differs in the ratio $H_{II}/H_I \approx 1.25$ for the two regions, contradicting the cosmologists' canon.

In the RFR¹ paper, an arbitrary division of the Universe into two was made, which has no evident physical significance. A non-cosmological redshift has been suspected for the centres of rich clusters of galaxies, like Coma^{2,3}, and the effect noted by Rubin *et al.* may be correlated with this.

To test this hypothesis we divided galaxies into two categories: first, those whose light does not encounter any important cluster of galaxies on its way to the observer (galaxies in region A), and second those situated behind or inside a cluster (region B).

Zwicky's Catalogue of Galaxies and Clusters of Galaxies (CGCG)⁴ supplies us with a suitable set of clusters of galaxies. We have limited our study to clusters classified 'near' ($V \leq 15,000 \text{ km s}^{-1}$) whose population was ≥ 500 (for 'compact' and 'medium compact' clusters) or $\geq 1,000$ (for 'open' clusters), so that their density of matter or of radiation is sufficiently high. We added the Virgo cluster which was not included in the CGCG because it is too near. Lastly, to include the possible influence of the gravitational and radiation fields of the clusters, we have included in region B an area around each cluster whose radius is double that of the cluster.

Distances of clusters are given by Noonan's list of published radial velocities⁵. For some clusters not included in that list, estimations were made with three different methods: first we estimated the luminosity classes of Sc and Sb galaxies in clusters^{6,7}; this immediately gave us a distance modulus. Second we checked our results for Abell clusters using Abell's method⁸. Third, the Uppsala catalogue⁹ supplied us with some other radial velocities.

Since the list of galaxies of Rubin *et al.*¹ is unfortunately not yet published, we used the following: 41 faint radio-sources identified with galaxies¹⁰. We used all the galaxies in the list within the intervals $13 \leq m_{\text{corr}} \leq 15.5$, and $4,800 \leq V \leq 20,000 \text{ km s}^{-1}$, and 77 compact galaxies with absorption spectra from Zwicky's Catalogue of Selected Compact Galaxies (CSCG)¹¹. The Rubin-Ford effect has already been confirmed for this type¹². We used the same radial velocity interval as for radio galaxies and revised the list with more simple criteria—we chose all compact galaxies within this velocity interval whose spectra show absorption lines but no emission lines other than [O II] $\lambda 3,727 \text{ Å}$, and which lie in the magnitude interval $13 \leq m_{\text{corr}} \leq 18$.

In spite of a few criticisms^{13,14}, the homogeneity of Zwicky's magnitudes within these limits, seems confirmed by the recent work of Paturel¹⁵, if a correction is made for declination dependence by the term $\Delta m_{\text{dec}} = 0.0025 \delta (d^\circ)$, and if the sample is limited to galaxies of given morphology. These factors do not interfere with our statistical calculations.

Table 1 Statistical results for the compact galaxies and the radio galaxies

Region		A	B
Radio galaxies	n	21	20
	m_{corr}	14.62 ± 0.139	14.36 ± 0.168
	$V_{\text{km s}^{-1}}$	$9,454 \pm 890$	$11,866 \pm 985$
	HM	1.01 ± 0.028	1.17 ± 0.022
Compact galaxies	n	53	24
	m_{corr}	15.88 ± 0.136	15.45 ± 0.194
	$V_{\text{km s}^{-1}}$	$11,147 \pm 582$	$11,376 \pm 809$
	HM	0.84 ± 0.026	0.94 ± 0.033

n is the number of galaxies, $\langle m_{\text{corr}} \rangle$ is the mean apparent corrected magnitude (see text), $\langle V \rangle$ is the mean radial velocity relative to the sun and, $\langle \text{HM} \rangle$ is the mean value of the Hubble modulus.

All apparent magnitudes are taken from CGCG or CSCG. Corrections are also made for galactic absorption by the formula:

$$m_{\text{corr}} = m - A_{\text{ps}} = m - 0.25 \csc \theta |b''|$$

The two samples of galaxies, with the outlines of region B are plotted in Fig. 1.

To study eventual abnormal redshifts, the best suitable parameter is the Hubble modulus (HM), defined:

$$\text{HM} = \log V - 0.2 m_{\text{corr}} = \log H - 0.2 M - 5$$

where H is the Hubble constant in $\text{km s}^{-1} \text{ Mpc}^{-1}$, and M , m_{corr} , V represent respectively the absolute magnitude, the corrected apparent magnitude and the radial velocity in km s^{-1} of a galaxy. With this parameter we can test either a variation of H (if the mean absolute magnitude has a sufficiently small dispersion) or an eventual deviation of the mean absolute magnitude, provided the distance-symbolic radial velocity ratio is regular enough. The results are given in Table 1, with standard errors.

We would like to make the following comments: first, we obtain the differences $\langle m_{\text{corr}} \rangle_{\text{B}} - \langle m_{\text{corr}} \rangle_{\text{A}} = -0.26 \pm 0.218$ and -0.43 ± 0.237 respectively for radio and compact galaxies. These results do not depend on the value chosen for the absorption coefficient, since similar differences are obtained with the non-corrected magnitudes: -0.31 ± 0.212 and

-0.33 ± 0.246 . Second, the differences of Hubble modulus are contrarily positive and highly significant:

$$\langle \text{HM} \rangle_{\text{B}} - \langle \text{HM} \rangle_{\text{A}} = 0.16 \pm 0.036 \text{ (radio galaxies)}$$

$$\langle \text{HM} \rangle_{\text{B}} - \langle \text{HM} \rangle_{\text{A}} = 0.10 \pm 0.042 \text{ (compact galaxies)}$$

Student's variable is $t = 4.4$ and $t = 2.4$ respectively, which are significant with a quasi-certitude of more than 99.9 per cent and 98 per cent. These results can be compared with the values obtained for the same samples in the case of the subdivision of Rubin *et al.*

$$\langle \text{HM} \rangle_{\text{II}} - \langle \text{HM} \rangle_{\text{I}} = 0.02 \pm 0.045 \text{ (radio galaxies)}$$

$$\langle \text{HM} \rangle_{\text{II}} - \langle \text{HM} \rangle_{\text{I}} = 0.08 \pm 0.043 \text{ (compact galaxies)}$$

These results suggest the following. First, Sandage and Tamman¹⁴ have suggested that the effect noted by Rubin *et al.* could be explained by a deviation from the normal distribution of absolute luminosity (that is, HM values) 'if the velocity field itself is inhomogeneous (clumped) and if the magnitude limits of the sample are very narrow'. This explanation does not seem available in our case. Indeed m_{corr} intervals of our samples are rather large and their redshift distribution is not clumped.

Second, for our samples, the differences of $\langle \text{HM} \rangle$ in our test are clearly accentuated and concentrated in comparison with those obtained with RFR division. As a consequence the RFR effect should be associated with the difference of distribution of clusters between the two regions of Rubin *et al.*

Third, since the distributions of our galaxies, in both region A and B, are rather uniform on the map of the sky (see Fig. 1), any explanation of the observed effect by a motion of the observer seems to be excluded. This excludes also the explanation by abnormal galactic absorption proposed, for example, by Hartwick¹⁶. Besides, absorption in the clusters cannot explain the effect, but on the contrary, would increase the difference.

Fourth, it has been suggested that clustering itself could give rise to systematic differences of $\langle \text{HM} \rangle$. Indeed absolute magnitude selection effects were reported by van den Bergh⁷. But the systematic difference he found between field galaxies and

cluster galaxies in the Virgo cluster (0.35 absolute magnitude), in the opposite direction to the ones we obtained, favour the underestimation of HM values in the clusters. Furthermore, to test the possible influence of clustering on our samples, we divided them into three regions: C (galaxies belonging to a cluster), A* and B* containing the galaxies which remain in the original A and B regions. We found (including standard deviations): compact galaxies: $\langle \text{HM} \rangle_{\text{B}^*} = 0.96 \pm 0.046$ ($n = 15$); $\langle \text{HM} \rangle_{\text{C}} = 0.86 \pm 0.040$ ($n = 12$); $\langle \text{HM} \rangle_{\text{A}^*} = 0.85 \pm 0.027$ ($n = 50$); and for radio galaxies: $\langle \text{HM} \rangle_{\text{B}^*} = 1.19 \pm 0.024$ ($n = 12$); $\langle \text{HM} \rangle_{\text{C}} = 1.11 \pm 0.033$ ($n = 12$); $\langle \text{HM} \rangle_{\text{A}^*} = 1.01 \pm 0.034$ ($n = 17$). It is seen that $\langle \text{HM} \rangle_{\text{B}^*} > \langle \text{HM} \rangle_{\text{C}} \geq \langle \text{HM} \rangle_{\text{A}^*}$ which suggests that the observed effect is not due to clustering.

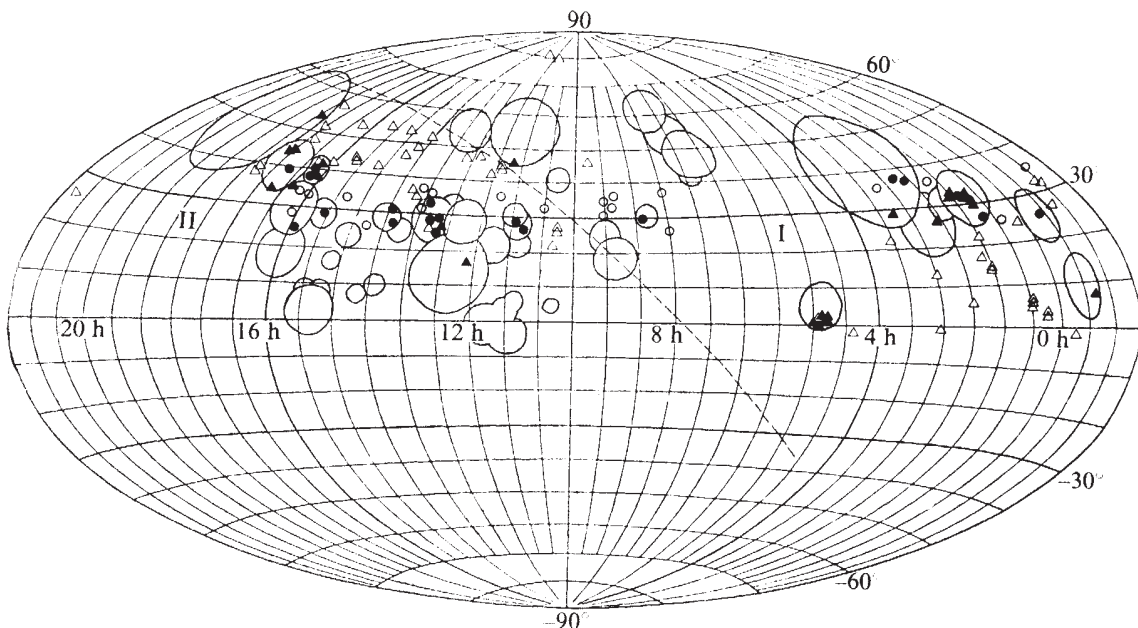
Finally, there remains, of course, the possibility that this phenomenon may be interpreted as Malmquist bias: for a magnitude limited sample, distant galaxies will be overluminous as compared to the mean. This could explain the observed differences of HM if the galaxies in region B are meaningfully more distant than in region A.

Such an interpretation is rather doubtful because we have checked that there is no significant difference of the mean geometrical distances between regions A and B ($L_{\text{B}} - L_{\text{A}} = 200 \text{ km s}^{-1}$, in symbolic radial velocity), so that the observed velocity difference cannot be interpreted in this way ($\langle V \rangle_{\text{B}} - \langle V \rangle_{\text{A}} = 2,412 \text{ km s}^{-1}$ for radio galaxies) and because Fig. 2 shows that differences of the mean HM exist within each narrow redshift band, so that the total average difference cannot be explained by a simple difference between the distribution of galaxies with distance in the two regions.

We must finally mention the possibility that the mean absolute magnitude of the observed galaxies is not constant over the sky and take into consideration possible uncertainties in the analysis. Contingent, however, on the verification of the effect with an improved sample, we tentatively propose two alternative interpretations:

(1) Light emitted by distant galaxies is redshifted when passing through clusters of galaxies (an effect which could be connected with 'tired light' theories^{17,18}) or (2) distant sources are more luminous when seen through intermediate clusters of galaxies, which could act, for example, as gravitational lenses.

Fig. 1 Distribution on the map of the sky of the clusters of galaxies and of our two samples of galaxies, in equatorial coordinates. The clusters are represented by their outlines. ○, radio galaxies in region A; ●, region B. △, compact galaxies in region A; ▲, region B. The dashed line is the border according to Rubin *et al.*



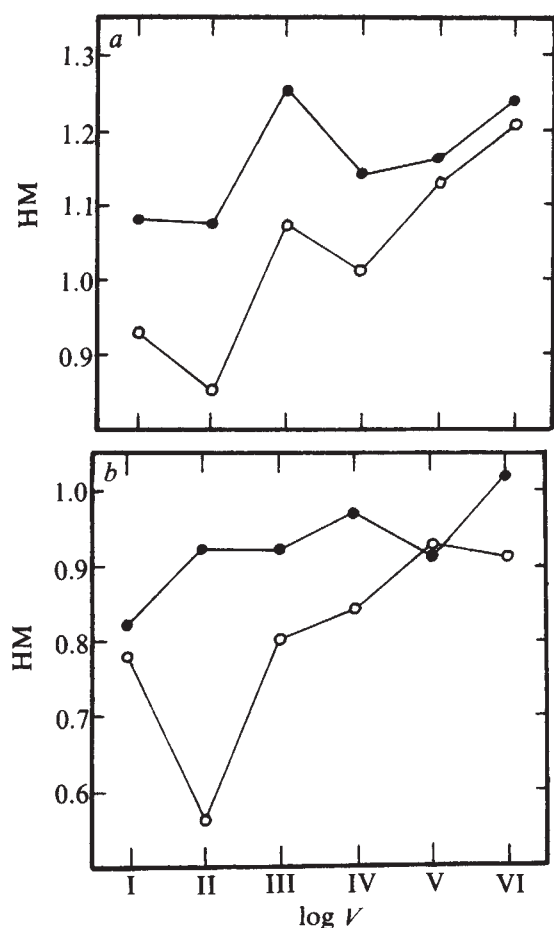


Fig. 2 ($\log V$, HM) diagram for (a) radio galaxies and (b) compact galaxies. The interval is divided into six narrow bands: I, 3.68–3.78; II, 3.78–3.88; III, 3.88–3.98; IV, 3.98–4.08; V, 4.08–4.18; VI, 4.18–4.30. In each band we calculate the mean of HM for region A ($\circ$) and region B ($\bullet$). The difference between the two regions persists for each band.

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Response of electrons in ionosphere and plasmasphere to magnetic storms

MEASUREMENTS of total electron content (TEC) using the Faraday polarisation-rotation and dispersive-group-delay techniques were carried out at Fort Monmouth, New Jersey^{1,2} (40.18°N, 74.06°W) using v.h.f. and u.h.f. beacon signals emitted by the satellite ATS-6. A comparison of TEC values obtained by both these techniques yields the total electron content of the plasmasphere. During the severe magnetic storms of September 15–16 and September 18–19, 1974, rapid increases with sudden onsets were observed in TEC as well as in plasmaspheric content, N_p . These increases were followed by long periods of low values of TEC and N_p .

Figure 1 shows one such variation of N_F (ionospheric content from Faraday rotation measurements), N_T (total content from dispersive-group-delay measurements), N_p ($\equiv N_T - N_F$, total plasmaspheric content) and τ ($\equiv N_F/N_{max}$, slab thickness, where N_{max} is the maximum electron density at Fort Belvoir, Virginia (37.9°N, 75.5°W) before and after a sudden commencement (SC). The K_p indices for that period are also indicated in the figure. While there was an abnormal evening increase in N_F , N_T , and N_p between 1930 and 0230 UT on September 14–15, a period of relatively low magnetic activity, the response of N_F , N_T , N_p and τ to SC is quite noticeable. For two hours after SC, N_F , N_T , and N_p increased rapidly; they oscillated for the next three hours, and then decreased rapidly to values well below the monthly average. After SC, τ also increased; it declined after 1930. Unfortunately, Wallops Island ionosonde data were not available for the period after ~2400, September 15. At the time of their maximum values (1545 UT, September 15), N_F and N_T were ~100% larger than their corresponding monthly averages. At the time of its maximum value (1730 UT, September 15) N_p was ~70% higher than its monthly average. During the period of depressed content values, N_F and N_T were at one point (1830 UT, September 16), ~45% of their monthly values, while N_p was ~21%.

Fig. 2 shows the variations of N_F , N_T , N_p and τ before and after SC, which occurred at 1434 UT (nearly the same time SC occurred in the previous storm) on September 18, 1974. Before SC, N_F and N_T were slightly lower or nearly equal to their monthly averages, while N_p was considerably below its monthly average. τ behaved similarly this time to its behaviour during the period preceding SC in the previous storm. For four hours after SC, N_F and N_T increased very rapidly; they then declined rapidly, and reached values below their monthly averages six hours after SC. N_F and N_T recovered quickly and attained values near their monthly averages. After 0700 UT on September 19, N_p increased rapidly after SC and reached its maximum ~15 min before that of N_F and N_T ; it then declined rapidly to values below its monthly average and remained there throughout September 19. N_p was not, however, as depressed this time as it was after SC in the days following the previous storm. For two hours after SC, τ increased rapidly; it then decreased to values not significantly different from its pre-SC values before both storms. At the time of their maximum values (1830 UT), N_F and N_T were ~120% larger than their corresponding monthly means and N_p (at 1800 UT) was 67% larger than its monthly mean.

The average behaviour of N_F and τ during geomagnetic storms at mid-latitudes has been discussed previously³. It was determined that N_F is characterised by an initial positive (a total ionisation enhancement relative to monthly mean values) phase lasting a few hours, followed by a negative phase (a total ionisation reduction) of a much longer duration, possibly lasting a few days. During the positive phase, τ is also enhanced because the relative increases in N_F are greater than those in N_{max} . As indicated by Figs 1 and 2, the storms of September 15–16 and September 18–19 are not atypical, as their behaviour was similar to the average behaviour.

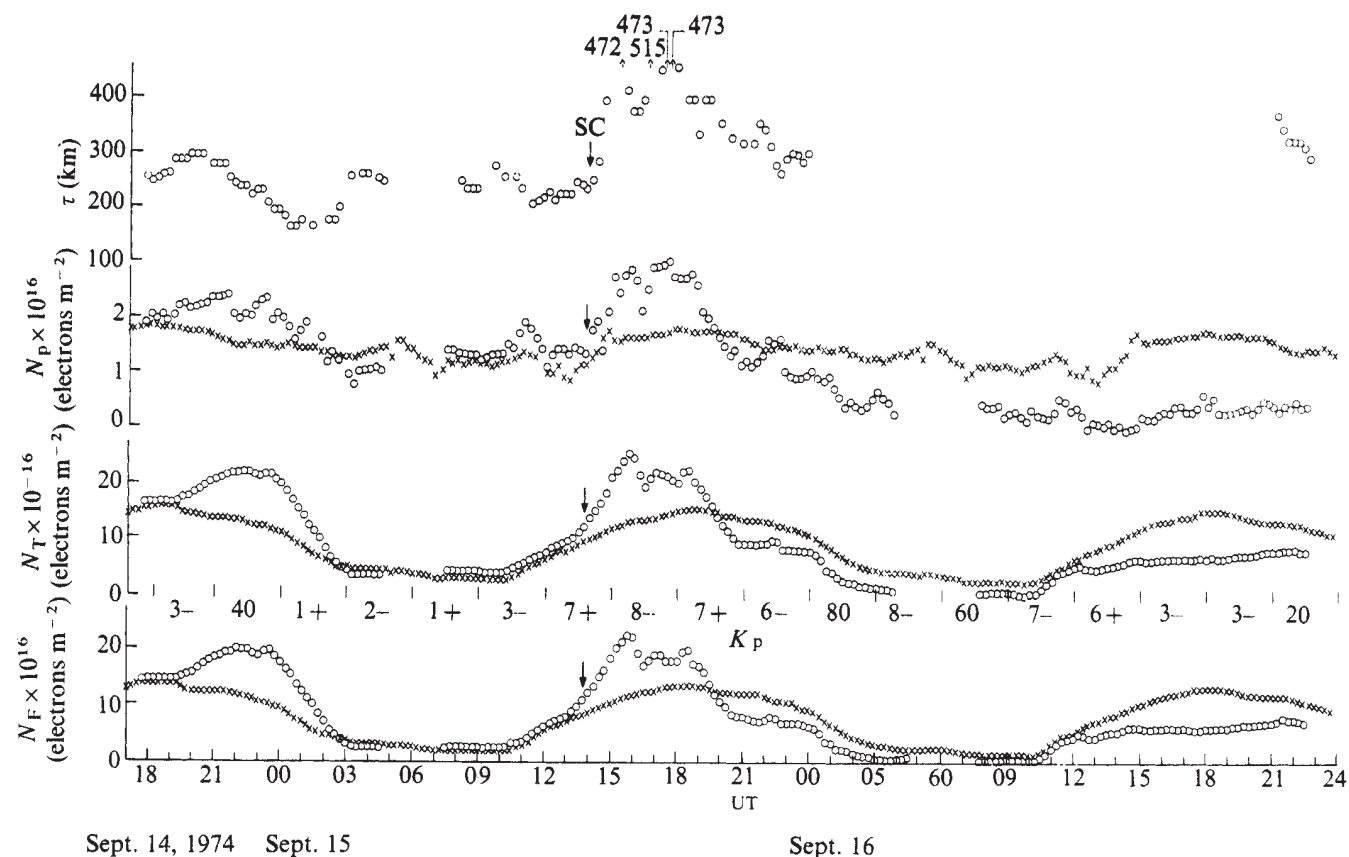


Fig. 1 Variation of N_F , N_T , N_P , τ and K_p from 1745 UT, September 14, 1974 to 2230 UT, September 16, 1974. Monthly averages of N_F , N_T , and N_P are indicated by $\times$.

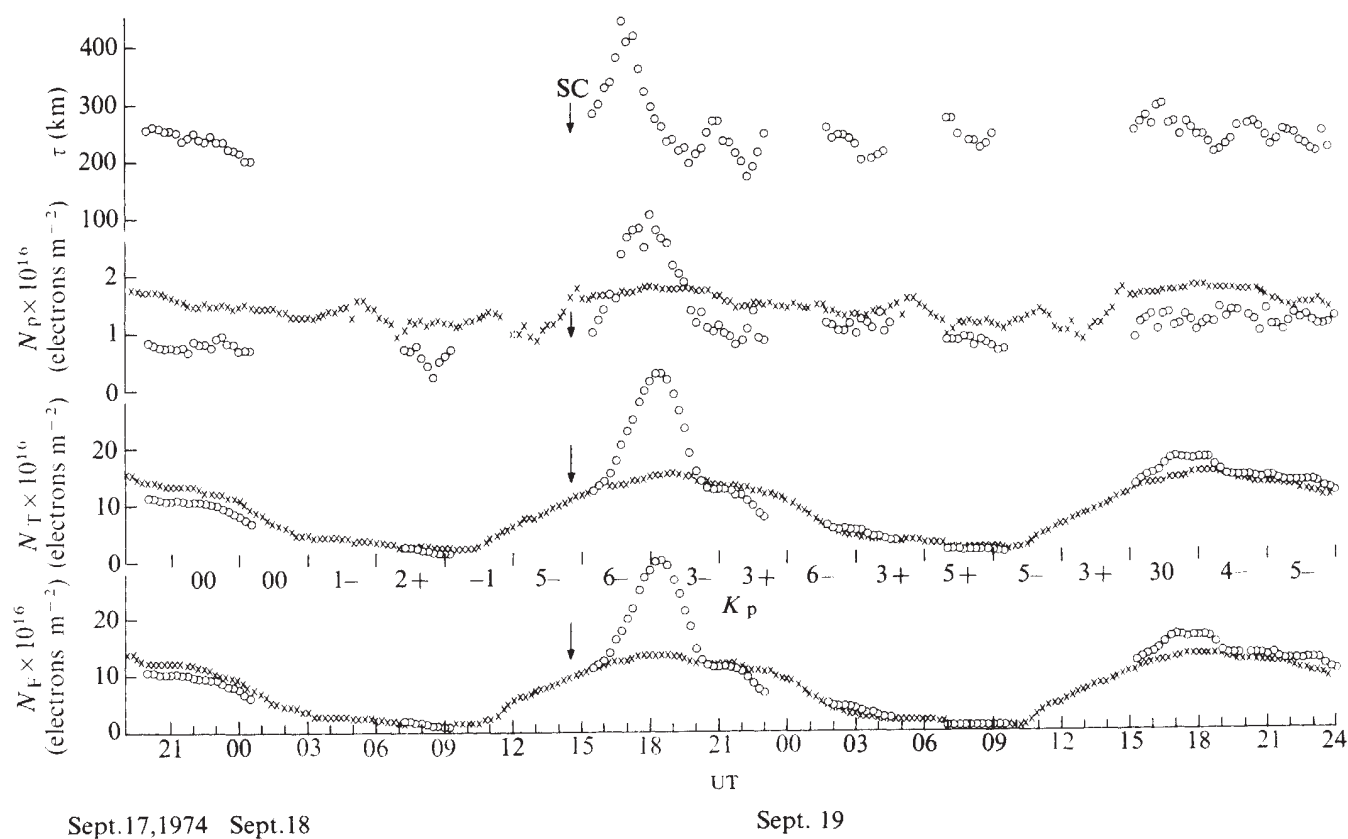


Fig. 2 Same as Fig. 1, but from 2000 UT, September 17, 1974 to 2400 UT, September 19, 1974.

The radio beacon experiment (RBE) of the ATS-6 yields new information regarding the behaviour of the plasmaspheric electron content, N_p , during magnetic storms. The RBE contains two relevant experimental modes. The first uses the Faraday rotation technique for determination of total electron content. Since the rotation is magnetic-field dependent, its value is weighted heavily in the vicinity of the Earth and it is assumed that the Faraday technique yields integrated content values (N_F) to heights of $\lesssim 1,500$ km. The second uses the dispersive-group-delay technique in which the phase of the modulation envelope between a carrier and its sideband is compared at two frequencies. Since the phase is insensitive to the Earth's magnetic field, this technique yields the number of electrons, N_T , along the entire path from the geostationary ATS-6 to the observer. The difference between the two, that is $N_T - N_F$, yields the total electron content above $\sim 1,500$ km, which is referred to as the plasmaspheric content, N_p .

The increase in N_p following SC tends to support the theory that increases in the total electron content during the positive phase are caused, at least in part, by the lifting of the F layer to regions of reduced recombination. Apparently, the upward fluxes of electrons after SC cause N_T to increase faster than N_F . This means that some of the electrons which were counted by the Faraday rotation technique had moved from the ionosphere to the plasmasphere. N_T increased, since the diffusing electrons had reached regions of reduced recombination. N_F increased at a slower rate, since, while recombination was reduced, some electrons had moved to the plasmasphere and were no longer counted. The net result was enhancement of plasmaspheric content.

The negative phase of the magnetic storm, which commenced about five to six hours after SC, occurred simultaneously with N_F , N_T , and N_p for both storms. As was previously suggested⁴, the heating effects of the neutral atmosphere, which disturb the normal production and loss processes in the ionosphere, were responsible for depletions of the ionospheric electron content. The plasmaspheric content, which is unaffected by normal production-loss processes, since these are ineffective at such heights, was very depressed compared to its corresponding mean monthly value. The plasmasphere is maintained by diffusive fluxes from the ionosphere. With the reduced values of the ionospheric densities, the plasmasphere was depleted by downward fluxes which seek to maintain hydrostatic equilibrium in the topside ionosphere.

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Simultaneous small and large scale irregularities in the ionospheric F region

THE study of scintillation-producing irregularities over Australasia indicates a continuous spectrum of scale sizes of ionisation clouds¹. Amplitude scintillations are most sensitive to small scale irregularities, peaking at 1 km transverse to the magnetic field^{1,2}. The presence of large scale irregularities could be detected from the measurements of total electron content (TEC), using the Faraday rotation or dispersive-Doppler technique, on v.h.f./u.h.f. transmissions from satellites orbiting the Earth. There is considerable evidence of large wave structures in the F region; the wavelength and period of large ionospheric disturbances are similar to those of large scale gravity waves^{3,4}.

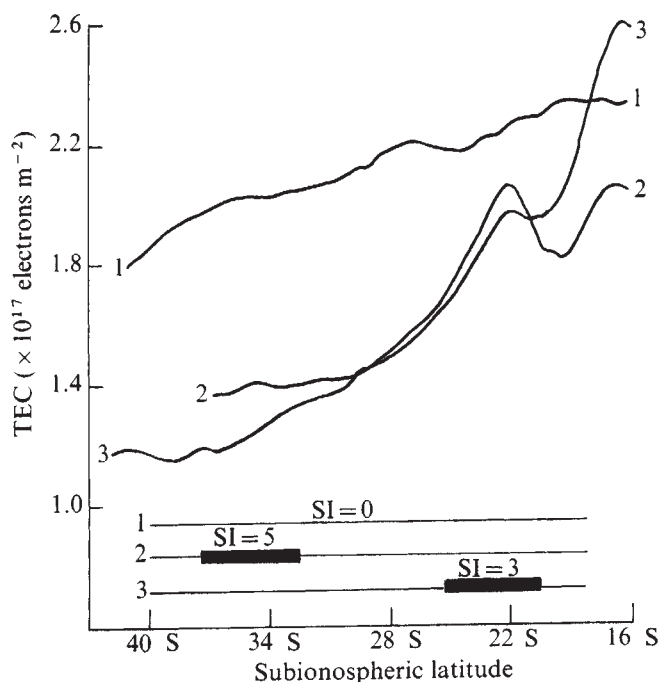


Fig. 1 An example of simultaneous occurrences of large fluctuations in TEC and scintillation activity.

Amplitude scintillation studies and TEC measurements have previously been conducted separately. This omission makes it difficult to cover the complete spectrum of irregularities since each of the techniques, used separately, has a limited spectral bandwidth. We therefore simultaneously recorded large and small scale ionisation structures in the F region. Amplitude scintillation studies were conducted using radio transmission from five US Navy navigational satellites (NNSS), at a frequency of 150 MHz. The recording station was in Brisbane (27°S, 152.9°E). Phase-locked receivers were used to receive radio signals from NNSS at two coherently related transmissions at frequencies 150 and 400 MHz. By employing a dual phase-locked receiver to monitor the phases of these two signals (and a suitable mixing technique to remove the vacuum Doppler) dispersive-Doppler phase shifts were measured. In turn, TEC computations were performed using the phase recordings. Thus, it was possible to record amplitude scintillations and TEC variations for the same satellite passes.

Figure 1 shows an example of simultaneous amplitude scintillations and TEC recordings obtained on November 24, 1973. Solid curves are TEC variations against subionospheric latitude, assuming the irregularity height of 400 km above the Earth's surface. The corresponding scintillation regions are indicated by horizontal bars. TEC and corresponding scintillations were recorded for NNSS passes with the following local times of the nearest approach: 1-1 (1756 LT), 2-2 (2046 LT) and 3-3 (2135 LT). The scintillation index (SI) varied from 60% (SI=3) to 100% (SI=5) modulation of the received signal (full definition of SI has been given elsewhere⁵). It can be seen that strong TEC variations were associated with pronounced scintillations during the second pass. The regions, where these two disturbances occurred were, however, largely different. A more typical situation is shown for the third pass when the large and small scale fluctuations occurred in a similar area.

We obtained 42 simultaneous TEC and amplitude records for November 1973 to January 1974. Forty per cent of the cases were recorded during quiet (SI=0) ionospheric conditions all with TEC amplitude (that is, percentage

deviation of TEC from the background ionisation) in the 0–5 range. During disturbed conditions ($SI=1-5$), 5% of the recordings had TEC amplitude 0–5, 38% had amplitude 5–10 (average 7.2) and 17% had amplitude 10–15 (average 13.6).

Relatively large TEC fluctuations were always associated with scintillation activity, and there were very few scintillations whenever TEC amplitude was less than 5% of the background ionisation. The latitudinal occurrences of TEC fluctuations and simultaneous scintillations were also examined. There were 10 cases for which there was complete spatial coincidence, 9 cases of partial coincidence and 5 of no coincidence at all.

In general, TEC fluctuations had a larger horizontal extent than scintillations. They exhibited a quasi-periodic structure with the wavelength lying in the range 300–600 km.

It seems, from the above considerations, that there is good temporal correlation between the occurrence of large and small irregularities in the ionosphere. The spatial coincidence, however, of both structures is not complete. This may indicate that gravity waves, which are responsible for the quasi-periodic structure of TEC fluctuations, do not necessarily produce small scale irregularities. The simultaneous temporal occurrence of both structures seems to be due to a common source. It has been suggested that the source of ionisation clouds, responsible for mid-latitude scintillations, is located in the evening sector of the auroral zone⁵. There is also strong evidence that large scale gravity waves, observed at mid-latitudes, are produced in the evening sector of the auroral zone⁴. Thus, it is likely that there is a bulk motion of ionisation patches causing scintillations are wave motion associated with TEC fluctuations. Both structures once originated in the auroral zone seem to propagate towards the equator.

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⁴⁰Ar/³⁹Ar dates for spreading rates in eastern Iceland

RADIOMETRIC dates for eastern Iceland have shown considerable scatter (see ref. 1 for a summary). This paper describes the results of the systematic dating of a stratigraphically continuous sequence of lavas (see Figure 1 for the sample locations; nomenclature follows ref. 2, from which further details can be obtained). As some of the lavas have experienced low-grade hydrothermal alteration³ the ⁴⁰Ar/³⁹Ar step-heating method was chosen, and the results of determinations on 18 samples from 11 sections are set out in Table 1.

Plateau ages are based on those steps whose apparent ages do not differ from the plateau mean by more than their estimated analytical error (s.d.). Means were calculated in two ways: weighting by the fraction of ³⁹Ar released during the step, and the inverse of the variance. As the difference in most cases was very small (suggesting the two methods are not independent) ages calculated using the latter method have been used in subsequent parts of this paper as it is the one in common use. All plateaux had at least four steps and comprised > 50% of

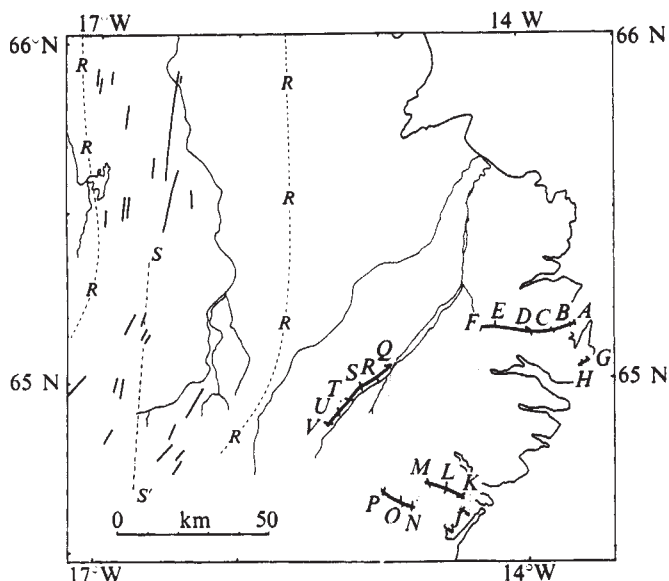


Fig. 1 Map of eastern Iceland. The large letters show the locations of the sections sampled by Dagley *et al.*², from whose collection the dating samples were selected. The dashed lines ---R--- denote the limits of the present active zone, and the heavy lines within this zone signify post-glacial fissure eruptions. The line S-S' approximates the centre of the active zone for the sections A-V. Map after ref. 2 and 15.

the ³⁹Ar released. Random deviations from the plateau occurred at both the lowest and highest temperature steps. In the case of K9 the initial step, involving 21% of ³⁹Ar, gave an apparent age of 143 ± 16 Myr, and though steps 2–5 gave a convincing plateau, this plateau age is significantly higher than that of K36 and appears anomalous in the succession; it therefore seems likely that some excess argon has been retained at all temperatures. As a saddle-shaped curve is a common feature of samples containing excess argon, of which the lowest part is sometimes horizontal enough to be considered a plateau^{4,5}, there is therefore the possibility that other samples which gave a high age for the initial step have a systematically high plateau age, but as the initial step of no other sample was so high or comprised so much ³⁹Ar, the error is considered negligible.

Fig. 2 Possible flow profiles. Three curves of the form of equation (2) for different values of k are given. Also shown are three observed horizons indicated by symbols: circles, Bodvarsson and Walker⁶ Fig. 3, section BB', profile between horizons H.T. and Gr.P; + and × respectively from right and left horizons of Fig. 2, Walker⁸. Curve A is assumed steepest flow profile (stratigraphic horizon) used to give probable minimum correction. Inset: Idealised section through lava pile. The source is to the left; the vertical scale is exaggerated.

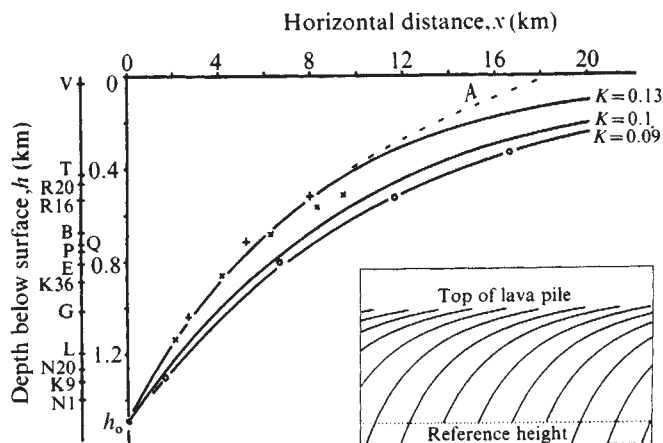


Table 1 Results of step-heating samples of lava

Section	Lava and core*	No. of plateau steps	% ^{39}Ar on plateau	Mean age I (Myr)†	Mean age II (Myr)‡	Total fusion age (Myr)§
V	18-1	4	85	2.0	1.96±0.44	
T	17A-2	5	100	3.1	3.31±0.72	3.1±0.5
R	20A-2	5	82	4.3	4.33±0.62	3.7±0.8
	16-2	4	77	5.3	5.26±0.15	6.4±0.4
O	13-1	4	90	5.8	5.81±0.16	6.1±0.6
P	14-1	5	80	5.7	5.68±0.17	
N	20-2	9	99	6.5	6.48±0.73	6.2±0.9
	1-1	4	60	7.8	7.83±0.11	7.0±0.3
L	33C-1	5	92	8.1	8.19±0.72	8.6±0.5
K	36A-2	5	86	9.6	9.58±0.41	9.5±0.5
	36A-2	6	91	10.2	10.11±0.27	10.4±0.4
	36A-1	5	87	10.2	10.18±0.22	10.6±0.4
	9-1	4	77	11.6	11.79±0.40	40.0±5.0
E	14-2	5	55	9.8	9.77±0.33	7.0±1.5
B	14-2	7	80	12.0	12.30±0.79	
	19-1	6	95	11.6	11.98±0.78	
G	19-1	4	80	13.2	13.19±0.10	
	21-1	4	75	12.9	12.92±0.14	

* Samples are arranged in stratigraphic order.

† Weighted by % ^{39}Ar released in each plateau step.

‡ Weighted by inverse of variance of each step age.

|| Deduced by summing all steps.

§ Estimated, as lowest temperature step not measured.

With this exception, the stratigraphic consistency of the dates shows the validity of the method.

Table 1 (and Fig. 3) shows that there can be no major time-gaps in the succession, and therefore affords no support for the large westward displacement of the spreading zone, postulated by Ward, which would result in a hiatus between sections P and Q⁶. Nor has a hiatus been found in the Western Iceland succession⁸. Table 1 also shows that evidence for glaciation is found in sections as old as P², ~ 5.5 Myr ago.

Calculations of spreading rates are not possible until allowance has been made for the fact that as a flow (or the group of flows which constitutes a section) has considerable lateral extent, it may be sampled over a range of distances from the spreading zone. In eastern Iceland the lava groups, on average, increase both their dip and thickness in a regular manner as they are followed westward⁹, so that, in principle, the distance of a flow from the axis of the spreading zone can be defined uniquely, though somewhat arbitrarily, by measuring it at some specified reference height within the lava pile (Fig. 2, inset). If a sample was collected at a different height, its distance from the spreading axis can be corrected to that height, provided the cross-sectional shape of a lava flow ('horizon') is known. In practice there are complications; the lava pile has been distorted, there are internal irregularities and erosion has removed its top. Thus, to make the correction it is necessary to know the position of the sample in the original pile, as well as the shape of the average horizon, both of which have to be deduced indirectly.

The height of the original top of the pile can be inferred from a study of the dyke swarms and the zeolite grade metamorphism, and the two methods give consistent results^{3,9}. A map showing the height of the original surface (taken as 600 m above the top of the analcite zone) has been published¹⁰; it varies from 0.7 to 1.7 km above present sea level.

The deduction of the shape of unmapped parts of the pile is not so easy, but depends upon the regularity of profile seen in exposed sections⁹. If it is assumed that a group of lavas thins away from the spreading zone according to

$$z = z_0 \exp(-kx) \quad (1)$$

where z and z_0 are the thicknesses of the group at two places separated by a distance x (measured horizontally perpendicular to the spreading zone), and if it is further assumed that the flows of the pile have the same profile over a significant distance

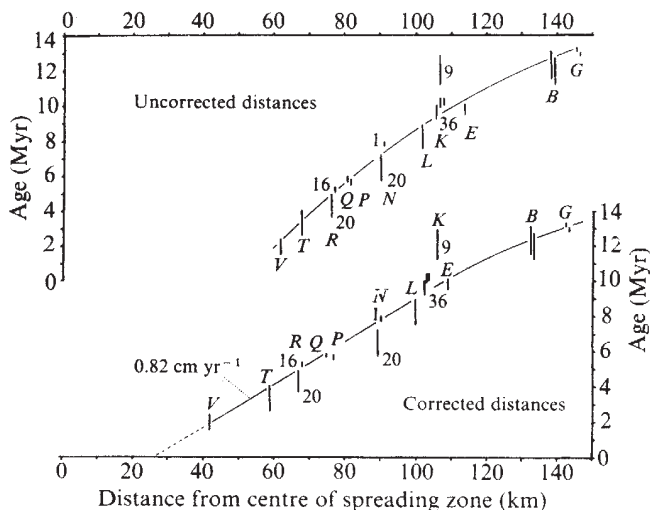
(Fig. 2, inset), then it necessarily follows that the horizons have the form

$$h = h_0 \exp(-kx) \quad (2)$$

where h_0 is an arbitrary depth that sets the zero of x (Fig. 2).

k can be estimated from reports in the literature in a number of ways; the rate at which the dip decreases in a vertical traverse of the pile $[(d/dh)(dh/dx)]$ is simply $-k$. Dagley *et al.*² state that "there is a regular upward decrease in the dip of a lava flow averaging 1° per 170 m increase in altitude", which, since it does not depend upon the altitude, supports equation (1), and yields $k = 0.102$. A second method depends on the dip observed at a specified height in the pile: that at sea level near section A (estimated at about 1.3 km below the original surface) is given as 6–8° (ref. 2). If a mean of 7° is chosen, equation (2)

Fig. 3 Age of samples against distance from spreading ridge. Distances have been measured perpendicularly to the line S-S' of Fig. 1; in the upper part these distances have been used unaltered, but in the lower part they have been corrected to a reference height 1.5 km below the surface of the pile, according to curve A of Fig. 2. Errors in distance are unknown but could be large. Samples are identified by section letter, plus number where necessary.



gives $k = 0.103$. Figure 2 shows a number of curves obeying equation (2) with $h_0 = 1.5$ km and different values of k . Also shown are some horizons described elsewhere (approximately perpendicular to the spreading axis), after correction to the inferred depth below the original surface. The actual profiles only approximate the curves from equation (2), and even then have a range of values of k from roughly 0.09 to 0.13. In addition, it should be noted that the mathematical model must breakdown as the profile approaches the surface, for the theoretical curves give some lavas extending to infinity, as implied in equation (1). Curve A of Fig. 2, an assumed extrapolation of the steepest of the three observed horizons, has therefore been used to make corrections, which are probably minimal.

Figure 2 is used to correct the sample positions to a common depth, taken as 1.5 km (the more obvious choice of the surface as reference height is precluded by the inadequacy of the model). The effect of the correction is most marked for the youngest and nearest-surface sections (R-V), and results in an increase in the apparent spreading rate over the time spanned by these sections. The errors on the largest corrections are more than proportionately large, as they depend on the estimation of the sample position relative to the original surface, the orientation of the line S-S' and on the shape of the flow horizon near the surface, where the model breaks down and where field observation is lacking. The correction for sample V18 is, however, unlikely to be < 18 km and is probably more, so that the spreading rate for the period from 10–2 Myr was probably uniform and not less than 0.8 cm yr^{-1} (half rate). At the present level of accuracy this is not distinguishable from the spreading rates on the Kolbeinsy Ridge to the north of Iceland (0.82 cm yr^{-1} on the eastern side¹¹) or the Reykjanes Ridge to the south [$\sim 1 \text{ cm yr}^{-1}$ (ref. 12)].

The corrected part of Fig. 3 extrapolates, for zero age, to a position about 30 km east of the centre of the present active zone, but within the active zone. This figure would be reduced if larger corrections were applied; on the other hand, if the distance from the spreading axis were referred to a reference height nearer the surface the figure would be increased. Before this intercept can be interpreted, a detailed understanding of the zone in which the lava pile is formed is needed; suggested models involve an active width of 50 km (ref. 13) or 70 km (ref. 14), and therefore, considering the large possible errors in Fig. 3, no significance is attached at present to the non-zero intercept.

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Direct conversion of graphite to diamond under static pressure

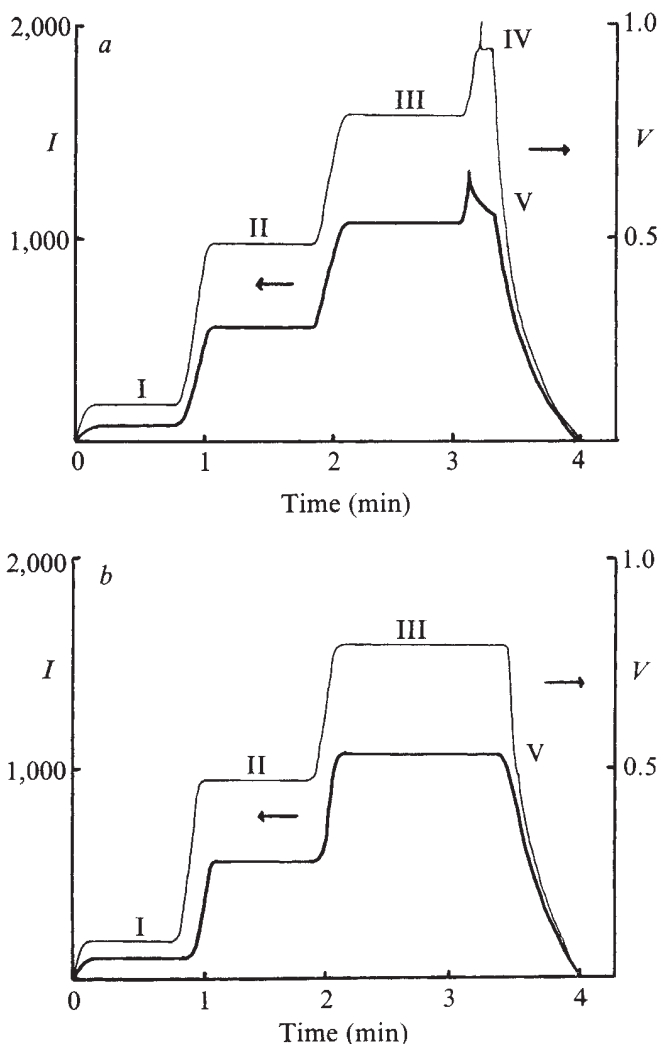
WE report the preparation of cubic type diamonds, by heating a graphite bar by passing an electric current directly through the specimen; we used a modification of the techniques developed in refs 1–4.

We obtained more than 60% of the theoretical yield. The transparent diamonds obtained were in the aggregated state, with a size of about 0.1 mm. The aggregate was found to be composed of a number of crystals with size of $\sim 10\text{--}20 \mu\text{m}$, which is larger than the size (200–500 Å) of diamond crystals synthesised by flash heating⁵.

A spectroscopic grade graphite bar, 3 mm in diameter and 7 mm in length (about 80 mg), was used. The specimen was subjected to a pressure of 140 ± 10 kbar, and then an electric current was passed through it. Figure 1 shows a plot of current-voltage against time: *a* is a typical case where diamonds were successfully obtained, *b* is a case where diamonds were not obtained.

The voltage was increased at fixed intervals and until step III, Ohm's Law was obeyed. When the voltage was increased to 0.95 V at step IV, a sudden increase in voltage and a decrease in the electric current were observed. The

Fig. 1 *a* and *b*, Current and voltage against time for spectroscopic graphite bar at 140 kbar. *a*, Voltage taken up to 0.95 V; *b*, voltage taken to 0.8 V.



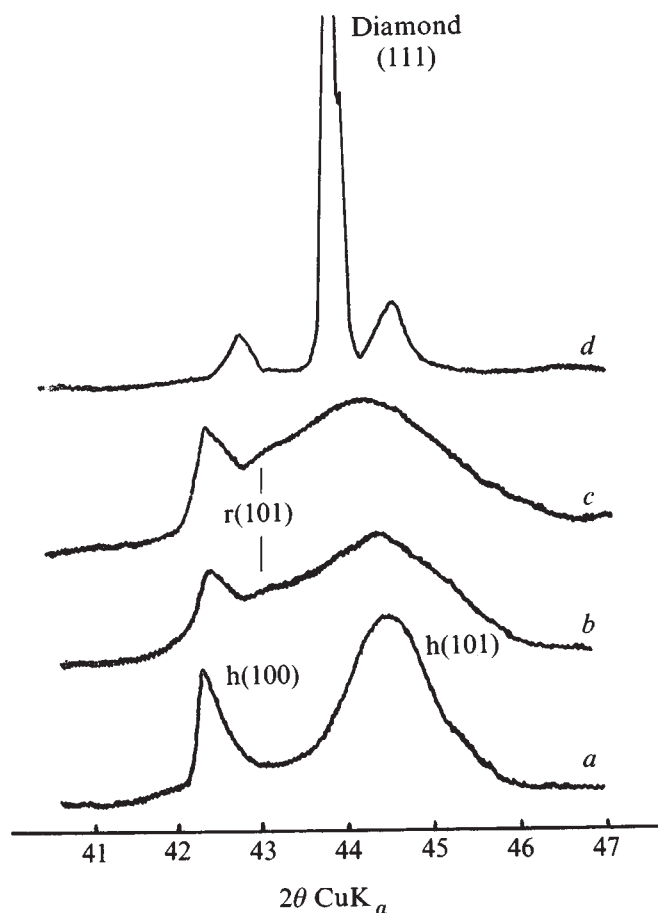


Fig. 2 X-ray powder diffraction patterns of the original graphite and the heat-treated specimens. *a*, Original graphite; *b*, $I, V = 0$ and $P = 140$ kbar; *c*, specimen obtained from the experiment recorded in Fig. 1*b*; *d*, specimen obtained from the experiment recorded in Fig. 1*a*.

voltage was then maintained at a constant 0.95 V, and the electric current decreased gradually for about 20 s. This phenomenon was similar to the observations of Bundy¹, but the conversion time of graphite to diamond in his experiment was said to be of 'millisecond' order, whereas the corresponding time in our experiments seemed to be of the order of seconds. Using the flash heating technique, homogeneous heating of the specimen should be obtained, with consequent rapid crystal transformation whereas using our resistance heating technique, a radial temperature gradient should be created. The core of the specimen would then be the first part to turn into diamond, and the transformation would subsequently propagate to the surface.

At step V, the heating power was purposely decreased. This was necessary to prevent the melting of the pressure medium, and contamination of the diamond.

If the voltage was increased to only 0.8 V, as shown in Fig. 1*b*, the transformation of graphite to diamond did not occur. The temperature at step IV in Fig. 1*a* may, therefore, be estimated, from the reaction diagram of Bundy⁵, to be 3,000–3,500 K.

The X-ray powder diffraction patterns of the original graphite specimen and the heat-treated specimens are shown in Fig. 2. By pressurising the original graphite, the hexagonal lattice of the graphite was distorted and partly converted to the rhombohedral lattice. Rhombohedral graphite was also obtained by grinding⁶. The rhombohedral lattice is stable on heating up to stage III and it was therefore concluded that the transformation to diamond in the metastable region of graphite must proceed through

a drastic change in the chemical bond. DeCarili and Jamison have also proposed that diamond is formed from the rhombohedral lattice of graphite⁷. Establishment of the mechanism of the transformation process will need, however, more detailed physico-chemical experiments.

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Language perception of 2-month-old infants shows effects of both innate mechanisms and experience

VOICING differences distinguish two or more stop consonants in nearly all the world's languages. It has been found¹ that voicing distinctions for stops in word-initial position can usually be characterised by differences in voice onset time (VOT), where VOT is defined as the time between the stop release burst and the onset of vocal cord vibration (voicing). For example, the English phonemes /p/ and /b/ differ in this way; voicing follows release in /p/, while voicing is approximately simultaneous with release in /b/.

Eimas *et al.*² found that American infants as young as 1 month old discriminated between the synthetically produced speech sounds, [b] and [p]. These infants failed, however, to discriminate between speech sounds which were separated by the same VOT difference as [b] and [p], but were members of the same phonemic category in English. (This result has been replicated for stimulus separations as small as 20 ms VOT and as large as 100 ms VOT³.) Thus, the infants, who do not yet produce these sounds, showed the same discrimination pattern as American adults; discrimination between phoneme categories was better than discrimination within phoneme categories⁴. Consequently, it has been proposed that the perception of voicing may be accomplished by innate perceptual mechanisms specifically tuned to perceive speech. To determine whether the discrimination of the English voicing distinction is independent of earlier linguistic exposure, I have investigated the discrimination of voicing onset by infants reared in a linguistic environment in which the English voiced/voiceless distinction is not used.

Kikuyu, a Bantu language spoken in Kenya, has only one labial stop, a prevoiced /b/, in which voicing substantially precedes articulatory release by an average value of 64 ms. The language distinguishes between prevoiced and voiced stops for other places of articulation, but Kikuyu has no stops in which voicing onset is substantially delayed relative to stop release as in the English voiceless, /p/, /t/, and /k/.

Discrimination was tested by a conditioned non-nutritive sucking habituation technique⁵. The infant sucked a blind nipple, which was attached to a pressure transducer. Criterial sucks whose amplitude exceeded an individually adjusted threshold (the baseline rate was approximately between 15 and 30 sucks min⁻¹) activated a counter and resulted in 1 s of sound presentation. The sound source was a tape loop with alternating syllables such as [ba] and silent intervals each 500

ms in duration. The sound source was gated in and out by a variable resistance device to minimise clicks. One or more criterial sucks⁻¹ continuously maintained the auditory signal at a comfortable listening level that produced few startle responses.

Typically, the infant's sucking rate increased over the baseline rate after a few min of pairing sucking and sound, presumably because of the reinforcing properties of the sound; eventually, however, the sucking rate decreased. This decrease has been attributed to habituation to the sound or its reinforcing value². When the sucking rate had decreased by $\geq 20\%$ of the rate in the immediately preceding minute for two consecutive minutes or had decreased by $\geq 20\%$, $\geq 0\%$, and $\geq 20\%$ respectively in three successive minutes, the sound was changed without interruption. The experiment was terminated 4 min after the sound shift. The rationale for this procedure is that if the sucking rate increases after the sound shift relative to a control condition with no sound shift, the sound shift must have been discriminated.

All stimuli were generated on the Haskins Laboratories' parallel resonance synthesiser and had the same formant frequencies and amplitudes as the stimuli used by Eimas *et al.* In all there were six syllables, a labial stop followed by the vowel [a] with VOTs of: -30 (prevoiced), 0 (voiced), 10 (voiced), 40 (voiceless), 50 (voiceless), and 80 (voiceless), which would be identified by English speakers as /ba/, /ba/, /ba/, /pa/, /pa/, and /pa/ respectively.

There were three experimental conditions with the following VOTs: (-30, 0), (10, 40), (50, 80) and a control condition in which pre- and post-shift sounds were the same, distributed equally over the six VOT values. The order of the three experimental conditions was counterbalanced across subjects as was the order of sound presentation within each condition. The control condition was either first or last. Each infant participated in all conditions.

Thirty-six periurban Kikuyu infants with a mean age of 63 d (s.d. = 12 d) participated in the study. All infants had heard only Kikuyu spoken in the home.

As in previous investigations⁶ using this technique with American infants, data were excluded for subjects who did not demonstrate adequate conditioning or habituation. Data were excluded if: (1) the pre-sound shift conditioning and habituation period was less than 4 min long and/or (2) if there were fewer than 15 sucks in either of the 2 min directly preceding the sound shift. The reason for the second criterion was that the infant had habituated too much, and consequently the stimulus would not be presented often enough to observe changes in its effect.

Analysis (unweighted means solution, repeated measures on two factors) of sucking rate in the 4 min directly preceding the sound shift indicated that all conditions were similar before the sound shift. (An analysis of variance revealed no significant effects of conditions or a conditions-minutes interaction.)

Discrimination was measured as an increase in sucking rate during the 2 min directly after the sound shift compared with the 2 min immediately preceding the shift. Analyses were performed using these difference scores. The analyses were *t*-tests, using split-significance levels ($\alpha = 0.05/k$, where $k = 3$). The analysis of these reduced data required the assumption of zero main effects and interactions for subjects. Since an analysis of the full data set showed that the between subject differences were near zero, this seemed to be a reasonable assumption. Both the (10, 40) and (-30, 0) conditions were different from the control condition ($P < 0.05$). The (50, 80) condition was not, however, different from the control condition, nor were there any significant differences among the three experimental conditions. Figure 1 shows the mean change in response rate for all four conditions.

Kikuyu infants thus made two linguistically relevant discriminations; they discriminated a [p] in which voicing preceded release from one produced with simultaneity of voicing and release, and they also discriminated a [b] produced with approx-

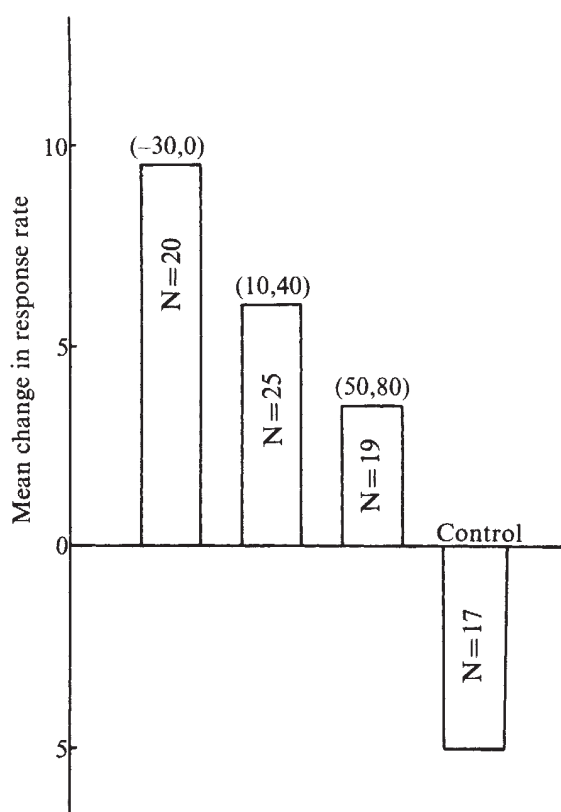


Fig. 1 Mean change in response rate per min as a function of experimental condition. The s. e. m. for the four conditions are: (-30,0) 3.24, (10,40) 2.77, (50,80) 3.58, and (control) 4.32.

imately simultaneous onset of voicing and release from a labial stop produced with substantial voicing lag [p]. It should be noted that adult Kikuyus showed the same discrimination pattern as the infants⁷. Thus, the ability to discriminate the voiced/voiceless distinction is not lost in the absence of relevant linguistic exposure, suggesting that this voicing distinction due to its psycho-acoustic properties is naturally discriminable.

The results of the present study differ from those of Eimas *et al.*, who found no reliable discrimination of the prevoiced/voiced distinction in American infants. Eimas³ reports that American infants discriminated the prevoiced/voiced distinction. The stimulus separation used (70 ms) was, however, much larger than the one used here, and was of the order of three times larger than required for adult phoneme discrimination in any language that has been studied.

The differences between the present study and that of Eimas *et al.* seem to be attributable to differences in American and Kikuyu linguistic environments. Kikuyu infants hear only one labial stop, a prevoiced /b/. But they do hear prevoiced stops contrasted with their voiced counterparts for other places of articulation. Thus, they are exposed to the acoustic cue or cues underlying the prevoiced/voiced distinction. On the other hand, American infants do not hear prevoiced stops in their linguistic environment. Thus, the results suggest that previous exposure to the prevoiced/voiced distinction in the language or perhaps exposure to the acoustic cues underlying this distinction is a prerequisite for discrimination of the contrast. On the other hand, the voiced/voiceless discrimination can be made in the absence of relevant linguistic exposure, since Kikuyu infants do not hear this voicing distinction used in their language. Thus, the results suggest that there is an interaction between nature and nurture; some phonetic or acoustic discriminations may be universal, whereas others seem to require previous relevant exposure.

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Behavioural fever in teleost fishes

THE ectothermic lizard *Dipsosaurus dorsalis* has been shown to exhibit a behavioural fever¹ when injected with killed *Aeromonas hydrophila*, a Gram-negative bacterium pathogenic to mammals, reptiles, amphibians and fishes, causing haemorrhagic septicaemia^{2,3}. The behavioural fever in lizards has been shown to have survival value⁴.

Mammals and birds use behavioural as well as physiological means to increase body temperature during a fever^{5,6}, whereas ectotherms such as lizards and fishes are limited largely to behavioural means alone^{1,7}. Evidence is accumulating that the hypothalamus controls thermoregulatory responses (both behavioural and physiological) in all classes of vertebrates, including fishes^{1,7-11}. Drugs such as pentobarbital which alter thermoregulation in mammals by acting on the hypothalamus¹² also affect behavioural thermoregulation in fishes^{13,14}.

Fever results from the effect of pyrogens on the hypothalamus^{15,16} by increasing the set points of the thermostat control centre⁷. Vaughn *et al.*¹ speculated that the behavioural component of fever might have evolved in early reptiles or even amphibians, and presented evidence for this in reptiles. We report here evidence that a similar behavioural febrile response exists also in fishes.

We tested eight bluegill sunfish, *Lepomis macrochirus* Rafinesque (120-155 mm standard length), and two large-mouth blackbass, *Micropterus salmoides* (Lacépède) (110-160 mm s.l.), matching the sample size of ten animals tested by Vaughn *et al.*¹, plus an equal number of controls. The normal thermoregulatory behaviour of these species has been extensively studied¹⁷⁻²¹.

We allowed each fish to thermoregulate in a device which enables a fish to control water temperatures, and thus body temperatures, by its movements between two chambers of different temperatures controlled by photo-transistor relays which monitor the position of the fish. The operation of this device has been described previously^{17,18}, having been based on an earlier design²⁰⁻²² which is similar in principle to that used by Vaughn *et al.*¹ for lizards. Recent work in our laboratory has shown that mean deep body temperatures in our device do not differ significantly from mean occupied temperatures, nor from mean water temperatures.

The fish were maintained in aquaria at 22-24 °C, on a light-dark (LD) 12:12 photoperiod. Fish were tested individually. Each fish was allowed an introductory period of 24 h in which to reach its preferred final temperature (~30 °C) and become acclimatised to it¹⁷. Then, mean occupied temperatures were monitored for a control period of 24 h. The fish was then injected intraperitoneally with 0.75 cm³ of fish physiological saline containing 4 × 10⁸ killed *A. hydrophila* cells, prepared as described by Vaughn *et al.*¹, and thermoregulatory performance was monitored

Table 1 Mean preferred temperatures (± 1 s.e.) before and after injection of *A. hydrophila* in ten fish

Species	s.l. (mm)	°C before injection	°C after injection	$\pm \Delta T$ (°C)
<i>Micropterus salmoides</i>	110	29.6 $\pm$ 0.3	31.9 $\pm$ 0.2	2.3
<i>Micropterus salmoides</i>	160	30.6 $\pm$ 0.3	32.3 $\pm$ 0.3	1.8
Grand mean (bass)		30.1 $\pm$ 0.5	32.2 $\pm$ 0.3	2.1
<i>Lepomis macrochirus</i>	120	31.9 $\pm$ 0.2	33.4 $\pm$ 0.2	1.5
<i>Lepomis macrochirus</i>	125	30.9 $\pm$ 0.2	32.4 $\pm$ 0.3	1.5
<i>Lepomis macrochirus</i>	130	31.6 $\pm$ 0.3	34.4 $\pm$ 0.3	2.8
<i>Lepomis macrochirus</i>	130	32.5 $\pm$ 0.2	37.2 $\pm$ 0.2	4.7
<i>Lepomis macrochirus</i>	145	27.9 $\pm$ 0.3	30.4 $\pm$ 0.2	2.5
<i>Lepomis macrochirus</i>	150	29.3 $\pm$ 0.3	32.2 $\pm$ 0.2	2.9
<i>Lepomis macrochirus</i>	150	30.8 $\pm$ 0.2	33.8 $\pm$ 0.3	3.0
<i>Lepomis macrochirus</i>	155	29.2 $\pm$ 0.2	31.4 $\pm$ 0.2	2.2
Grand mean (bluegill)		30.5 $\pm$ 0.6	33.2 $\pm$ 0.7	2.7
Grand mean (all fish)		30.4 $\pm$ 0.5	33.0 $\pm$ 0.6	2.6

for a further 24 h. An equal number of matched control fish were similarly handled, and injected only with fish physiological saline.

Hourly occupied temperatures were tabulated for the 24-hour periods before and after injection of each fish. The mean occupied temperatures were calculated, as were grand means (means of means) for each species and for all the test fish (Table 1). All bacteria-injected fish showed a significant increase (*t* test, 0.05) in preferred temperature: mean temperature increase (ΔT) was 2.6 °C for all test fish, 2.7 °C for the bluegills and 2.1 °C for the bass. The controls showed no increase.

We conclude that fish exhibit a behavioural fever, as do lizards, under the influence of bacterial pyrogens. This is probably due to a direct influence on the hypothalamic thermoregulatory centre, although direct evidence for this mechanism in fish awaits further neurophysiological study. Our data support the hypothesis that the neural mechanism for the control of thermoregulation, in the preoptic hypothalamus, had a common origin early in the evolutionary history of vertebrates^{1,7-11}. Fever as an adaptive response to infection²⁻⁴ may have similarly had its origin early in vertebrate history, even before the evolution of a terrestrial mode of life.

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Gravity as the sole navigational aid to the newborn quokka

ALTHOUGH there is general agreement on the sequence of events at birth for several marsupial species¹, it is still not known how the newborn joey navigates on its journey from the mother's cloaca to her pouch. Frith and Calaby² have postulated that, at birth, the red kangaroo (*Megaleia rufa*) "... has large nostrils and presumably a well developed sense of smell which probably plays a major part in its location of the pouch". On the other hand, Hartman³ has suggested that the newborn opossum (*Didelphis virginiana*) was "negatively geotropic" though he has now modified this view considerably⁴, as histological studies have revealed that the vestibular apparatus of the newborn animal is undifferentiated and therefore non-functional^{5,6}. To investigate whether one of these mechanisms^{2,4} could account for the behaviour of the newborn quokka (*Setonix brachyurus*) we have observed parturition in this macropodid marsupial.

Female quokkas mate soon after giving birth and such animals often develop a blastocyst in the state of diapause in the uterus. Removal of the pouch young causes this blastocyst to resume development so that a birth occurs about 26 days later⁷. In March 1974 and 1975 pouch young were removed from a total of 14 quokkas obtained from a wild population on Rottnest Island, Western Australia. The animals were transferred to a controlled environment 22 d later, then observed continuously. In all, seven animals gave birth; four births were seen and one was recorded in detail on videotape.

Our observations of these births confirm those of Tyndale-Biscoe⁸. In spite of intensive antepartum grooming of the pouch and cloacal region, there is no evidence of a path made by the mother to facilitate the passage of the joey from the cloaca to the pouch. The mother's position during birth was such that the abdominal wall was almost vertical and the lower lip of the pouch pouted outwards. Observation of the actual entry of the joey into the pouch was difficult because of the stance of the mother but on one occasion the joey seemed merely to tumble in over the lip.

After a birth in 1974 the joey was retrieved as soon as it had reached the pouch. The mother was then held vertically, head up, and the joey was placed on the upper (anterior) lip of the pouch. During the next 3 min it climbed about 3 cm upwards away from the pouch. The mother was then turned upside down. The joey faltered and eventually turned completely around so that its head was again pointed upwards, towards the pouch. It then became entangled in the mother's fur and made no progress. When the mother was returned to the head up position the joey reversed its direction again and progressed away from the pouch. It was then removed from the mother's chest and replaced near the cloaca, whence it succeeded in making a second journey to the pouch.

On the occasion of the videotaped birth in 1975 the mother was removed from her cage and held almost vertical, with her right side slightly lower than her left, while the newborn joey was still making its initial journey to the pouch. This led to its direction changing slightly so that it passed by the pouch; it did not falter but continued to climb to the mother's chest, leaving a distinct path, about 1 cm deep, in the fur. The mother was then turned to the head down position and, as before, the joey slowly turned around, this time without becoming entangled in the fur,

and made progress back towards the pouch. The joey was not handled during this experiment.

These observations suggested that the newborn quokka has a righting reflex; we found that it could also turn over when placed on its back. But older pouch young, up to the age of 65–70 d, no longer retain this ability, and histological sections through the head of a newborn joey support the conclusion that the vestibular apparatus is undifferentiated^{5,6}. It seems to us that this transient righting reflex could be mediated by muscle stretch receptors, particularly in the neck.

These experiments confirm the negative geotropism of the newborn quokka and reveal no other mechanism directing its movement towards the pouch. In the second experiment, in particular, the joey passed within 1 cm of the pouch opening without hesitating in its upward climb and it is clear that chemotaxis can be discounted in this case.

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Impaired acquisition of a passive avoidance response after lesions induced in the locus coeruleus by 6-OH-dopamine

Crow¹ and Kety² have advanced the hypothesis that the fine network of noradrenaline-containing nerve terminals which innervates the cerebral cortex³ is a necessary component of the mechanism of learning. In particular, it is suggested that these neurones deliver a "results of action"⁴ signal which registers the successful outcome of a particular behavioural sequence. The noradrenaline released in the cortex is believed to interact with a transient synaptic change resulting from recent neural activity (the 'short-term trace'), to initiate the long term synaptic changes presumed to underlie learning. Various authors have suggested the existence of such a "confirming reaction"⁵, "results of action"⁶, or "now print"⁶ mechanism. The diffuse distribution of noradrenergic terminals in the cerebral cortex would allow this system to deliver a generalised message of the type required. Ungerstedt⁷ has demonstrated histochemically that the cortical noradrenergic terminals originate from cell bodies of the nucleus locus coeruleus in the dorsal pontine tegmentum, and this has been confirmed in biochemical studies^{8,9}. The concept of the coeruleo-cortical noradrenergic system as a reinforcement pathway is supported by the observation that rats with electrodes in the region of the locus coeruleus can be trained to self stimulate^{10,11} and this behaviour is accompanied by increased noradrenaline turnover in the ipsilateral cortex¹².

The prediction that bilateral lesions of the locus coeruleus would impair learning capacity^{1,2,13} has been tested in a runway⁸ and a two-choice discrimination learning test¹⁴.

Since moderately extensive lesions of the locus coeruleus did not impair acquisition in this latter task it has been argued¹⁴ that the apparent impairment in runway performance either does not constitute a learning deficit, or is not representative of a general impairment of learning capacity. By contrast, in an avoidance situation, Stein *et al.*¹⁵ have shown that the learning deficit which can be demonstrated following administration of diethyldithiocarbamate (DDC), a drug which depletes noradrenaline stores by inhibiting dopamine- β -hydroxylase, can be reversed by intraventricular administration of noradrenaline. These findings suggest that the integrity of certain central noradrenergic pathways is necessary for learning in this situation. Here we have investigated whether this deficit in avoidance learning is a consequence of impaired transmission in the coeruleo-cortical noradrenergic system.

Seventy-nine female Sprague-Dawley rats (initial weight 150 g) were divided into an untreated control group ($n=24$); a group in which the coeruleo-cortical system was damaged by a stereotaxic injection of 6-OH-dopamine (6-OH-DA) (16 μ g in 4 μ l, bilaterally over 4 min) delivered through burr holes just anterior to the locus coeruleus 3 weeks before testing ($n=23$); a group with burr holes but no injection ($n=23$); and a group treated with DDC 300 mg kg^{-1} between the first and the second trials of the training procedure ($n=9$). The time taken for each animal to step off a platform on to the electrifiable grid floor of the test chamber was recorded by contact switches in each of five test sessions. The first two trials were separated by 2 h, and after 1 h all animals were given either DDC or saline subcutaneously (Fig. 1). On the third trial, animals were shocked (4 mA, scrambled across the grid, for 3 s) as they stepped down, and on the fourth (1 min later) and fifth (3 d later) trials the effects of this experience on the animals' subsequent step-down time was tested. With this experimental design it is likely that dopamine- β -hydroxylase inhibition in the DDC-treated group is present at the time

Fig. 1 The experimental design DDC, 300 mg kg^{-1} by subcutaneous injection.

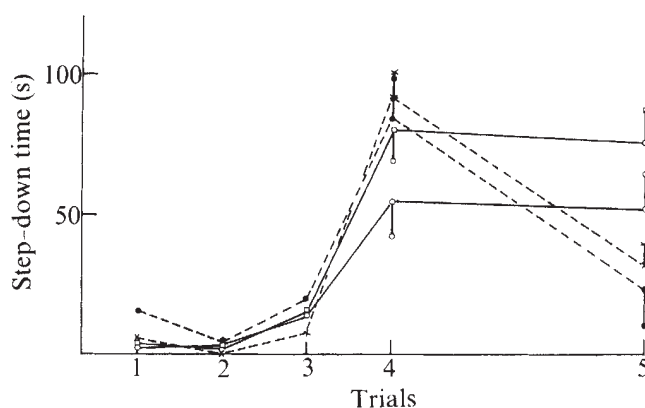
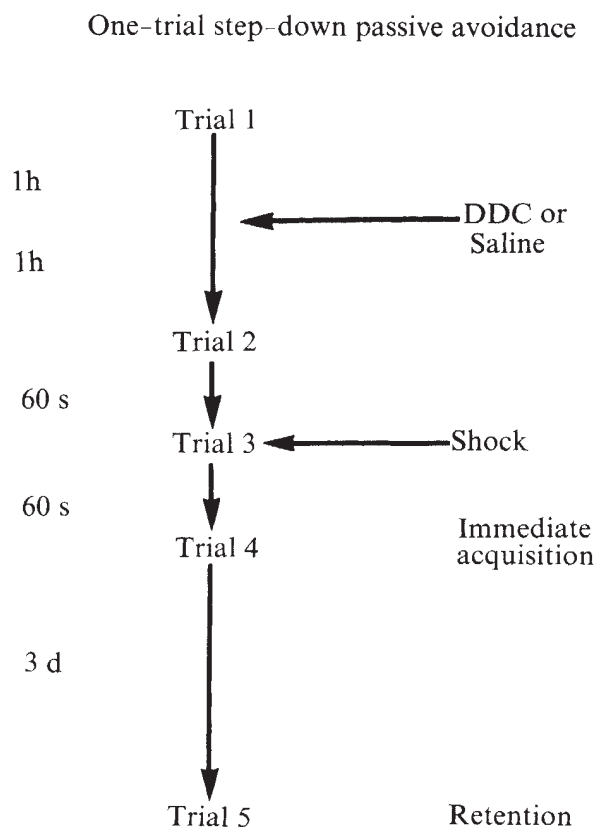


Fig. 2 Mean step-down times (s) on trials 1-5 in groups of rats treated with 6-OH-DA 3 weeks before testing ($\times$), and DDC (300 mg kg^{-1}) between trials 1 and 2 ($\bullet$), compared with untreated controls ($\square$) and a group of rats who had burr holes drilled in the skull ($\circ$). All animals received foot shock on stepping down on to an electrifiable grid floor on trial 3. The change in step-down time between trials 3 and 4 (t_4-t_3 in Table 1) represents the effects of shock on the animal's behaviour after 60 s (immediate acquisition). The comparison between trials 4 and 5 (t_5-t_4) is an index of the animal's ability to retain the avoidance response after 3 d (retention). Bars indicate s.e.m.

of the learning experience (trial 3) and at the time of the immediate acquisition test (trial 4), but is not present on trial 5 (ref. 15).

Both the 6-OH-DA- and DDC-treated rats showed normal immediate acquisition of the avoidance response as assessed by an increase in step-down time between trials 3 and 4 (Fig. 2 and t_4-t_3 in Table 1). Neither group differed significantly from the untreated controls, although the 6-OH-DA-treated group somewhat surprisingly performed significantly better than the burr-hole group (Table 1). Compared with both the untreated controls and the burr-hole group, the two experimental groups showed a failure of retention after 3 d, as shown by the differences in step-down time between trials 4 and 5 (t_5-t_4 in Table 1). Compared with the two control groups, the 6-OH-DA-lesioned and DDC-treated rats both showed a significant ($P<0.01$) decrease (t_5-t_4 in Table 1) in time spent on the platform. Activity levels measured in an Animex instrument (LKB Farad) showed no significant differences between the 6-OH-DA-lesioned rats, the group with burr holes and the controls (Table 2). Subsequently, the brains of the rats in the 6-OH-DA-lesioned group were removed and the cortices dissected out and assayed for noradrenaline using a spectrofluorimetric technique. The mean cortical noradrenaline level in the 6-OH-DA-lesioned group ($n=23$) was 93 ± 12.6 (s.e.m.) ng g^{-1} , approximately 15% of that present in a sample of the control group, 611 ± 68 ng g^{-1} ($n=4$).

Since immediate acquisition in these rats is unimpaired,

Table 1 The increase (t_4-t_3) in step-down time immediately following foot shock on trial 3 (immediate acquisition) and the change (t_5-t_4) in step-down time after a subsequent 3-d interval (retention)

	<i>n</i>	Immediate acquisition t_4-t_3 (s $\pm$ 1 s.e.m.)	Retention t_5-t_4
Untreated controls	24	63.7 ± 9.5	5.2 ± 10.2
Burr holes only	23	43.6 ± 9.7	-0.9 ± 16.6
6-OH-DA lesions	23	$83.2 \pm 9.0^\dagger$	$58.5 \pm 10.4^*$
DDC 300 mg kg^{-1}	9	63.6 ± 12.2	$60.8 \pm 17.0^*$

* $P < 0.01$ compared with untreated controls and burr-hole group.

† $P < 0.01$ compared with burr-hole group.

Table 2 Small and large movements measured in an Animex instrument over a period of 5 min

	<i>n</i>	Controls 24	Burr holes 23	6-OH-DA lesioned 23
Movements	Large	54 ± 4	64 ± 6	55 ± 6
	Small	154 ± 6	162 ± 10	166 ± 9

it seems unlikely that the deficit is due to impaired sensory or motor capacity; more probably it represents a defect in the capacity to form a long lasting record of previous experience. Moreover, since an identical impairment is observed after both administration, at the time of the learning experience, of drugs known to impair the synthesis of noradrenaline and after neurochemical lesions of a central noradrenaline-containing pathway, it seems likely, as Stein *et al.*¹³ suggest, that the deficit results from impaired central noradrenergic transmission at a critical stage in the learning process. Our results are compatible with the hypothesis that the system of noradrenergic neurones arising from the locus coeruleus is necessary for initiating the transition from a short to a long term memory trace, and that the integrity of this system is necessary for this transition to occur, at least in an avoidance learning situation.

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and took as his datum that the correlation between mates could be described by a bivariate normal distribution with coefficient μ . He found that if females with standardised value x occur in the population with frequency N and males with value y have frequency M , then x - y pairs occur with frequency $MN(1 + \mu xy)$. In populations with random mating the corresponding frequency is MN , so that, if $\mu > 0$, Fisher's assumption implies a higher frequency of pairs when both partners have above average or below average values, and lower when one has above and the other below average values, compared with the randomly mating population.

Malécot⁴ started from this implication and derived the distribution for one locus, $f_0 = (q^2 + \lambda qp)$, $f_1 = 2(1 - \lambda)qp$, $f_2 = (p^2 + \lambda qp)$, which is of the same form as Wright's, the only difference being the replacement of Wright's fixation index by Malécot's "constant of homogamy" λ . If the genotypes are given arbitrary values 0, 1, 2, respectively, the mean and variance of Malécot's distribution are $m = 2p$ and $s^2 = 2(1 + \lambda)qp$, respectively. Also, the deviations from the mean of the genotypes are $d_0 = -2p$, $d_1 = q - p$, and $d_2 = 2q$, respectively.

Consider a population which starts with Malécot's distribution in both sexes and assume that the frequencies of x - y mating pairs are given by

$$f_{xy} = f_x f_y (1 + \mu d_x d_y / s^2)$$

where $x = 0, 1, 2$ and $y = 0, 1, 2$. Then it can be shown that, given the usual Mendelian assumptions, the distribution of genotypes among offspring is again that of Malécot, provided that μ and λ are related by $\mu = 2\lambda / (1 + \lambda)$. Furthermore, it is found that, for all mating frequencies to be non-negative, μ must be restricted to the range $(-1/p^4, 1)$ if $q \leq p$, and $(1 - 1/q^4, 1)$, if $q > p$.

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Generalisation of the Hardy–Weinberg law

THE distribution of genotypes $q^2aa + 2qpaA + p^2AA$, commonly called the Hardy–Weinberg law, has been recognised at least since 1908. It gives the frequencies of genotypes in a randomly mating population with respect to a single locus maintaining two alleles a and A with respective frequencies q and p . A generalisation given in 1922 by Wright and discussed in his book¹ gives the distribution

$$(q^2 + Fqp)aa + 2(1 - F)qpaA + (p^2 + Fqp)AA$$

where F , the "fixation index" is the correlation between pairs of gametes uniting to form zygotes.

Here we point out that a distribution of the same form as Wright's is maintained in equilibrium by a pattern of assortative mating devised in 1918 by Fisher² and elaborated in 1939 and 1948 by Malécot³.

Fisher was concerned with continuously varying characters

Cross-modal transfer in the chimpanzee

TASKS of cross-modal performance assess the capacity to recognise stimulus-objects with one of the senses when they have previously been experienced only with another. In a number of recent publications^{1–3}, it has been shown that apes succeed at such tasks, in marked contrast to the negative or weak effects previously found for monkeys. The training procedures used with the apes were, however, new¹, and had not been tried with monkeys, so that it remained possible that monkeys would perform as well as apes with these procedures^{4–6}. Monkeys have been shown capable of cross-modal performance in two different methods of training (ref. 6, and M. J. Jarvis and G. Ettlinger, unpublished) one of which was designed to follow the principles used successfully with apes. We here describe the performance of three chimpanzees on cross-modal tasks of a kind which have in the past not yielded positive results with monkeys: our findings with the chimpanzees are unclear, or at least not certainly positive.

We assessed cross-modal transfer in three male chimpanzees (*Pan troglodytes verus*) weighing 16–20 kg, using procedures which have been described in earlier reports on monkeys^{7–9}. The sequence of training is shown in Table 1. After preliminary

adaptation to making two-choice simultaneous discriminations by pushing lids of food boxes in a WGTA in the dark and light, the apes were trained on five transfer tasks. Each task was presented to one sense until the animal reached a standard level of performance (45 correct responses in 50 successive trials for the initial four tasks, 160 correct responses in 200 trials for task 5); and then the same pair of objects was presented to the other sense, either with the same object rewarded as before (non-reversal) or with the previously unrewarded object now rewarded (reversal). In tactile training the objects were palpated directly in total darkness. Performance was monitored with a video system sensitive to infrared light. For visual training the objects were mounted on similar lids of food boxes, but a small handle was put on the lid in front of the object. These handles had to be used in the preliminary colour discrimination (as the colours were flat papers) and subsequently the handles were used (instead of the objects) for pushing the lids on all visual trials except three. The test objects are shown in Fig. 1; 40–60 trials were given daily, five days each week, with two peanuts as reward for a correct response. The poor learning scores may reflect concurrent daily training in three other investigations: after completing its training for this experiment early each morning each ape was later trained on the same day on memory, sorting and cross-modal recognition tasks. (Observations did not suggest that ≈ 200 trials were too many for each animal on each day. We prefer, with Warren¹⁰, to suppose that an ape does not learn more readily than a monkey—and may learn more slowly.)

The learning scores are shown in Table 1. If the animal failed to relearn within 300 trials in the second sense, training was discontinued. Cross-modal transfer could become manifest in either (1) higher learning scores in the second sense on tasks involving reversal of reward than on non-reversal tasks; (2) lower savings scores for the second sense at reversal rather than non-reversal tasks, and/or (3) a greater number of errors in the initial ten trials in the second sense for reversal than non-reversal tasks. The mean learning scores in the second modality

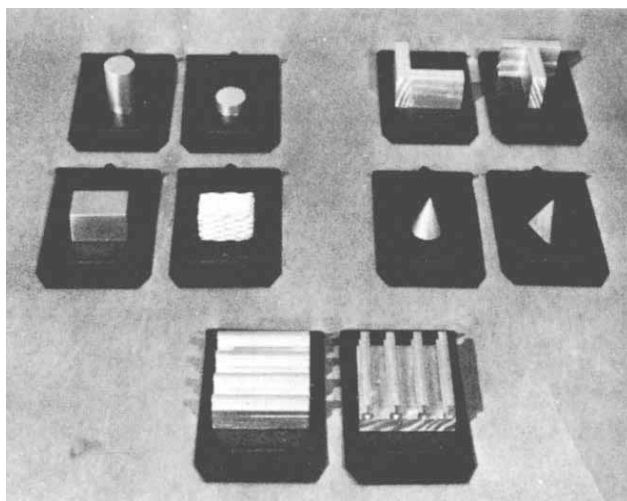


Fig. 1 Stimulus objects used for the visual versions of five transfer tasks. The objects were painted silver and set back on the lids of the food container behind small handles. The handles were used by the apes to push the lids so that no tactile information was gained during visual training in the light. For the tactile versions identical objects were painted matt black and fixed closer to the animal on another set of lids. The animals touched the objects directly to push the lids in the dark, and never gained visual experience of the objects. The tasks are identified in Table 1.

are 115 trials for 8 reversal and 120 trials for 6 non-reversal tasks. The mean savings are calculated from

$$\frac{1^{\text{st}} \text{ hand} - 2^{\text{nd}} \text{ hand}}{1^{\text{st}} \text{ hand} + 2^{\text{nd}} \text{ hand}}$$

to be -18.5 for 8 reversal and $+7.2$ for 6 non-reversal tasks. The mean errors in the first ten trials of the second modality are 6.4 errors for 8 reversal and 4.5 for 6 non-reversal tasks. After training on the transfer task 5 the procedure of Moffett and Ettlinger⁸ was adopted for Algie and Blue: the apes were given 80 trials at random in the light and dark with the opposite orientations rewarded exactly as during learning in each modality (that is, choice of sagittal rewarded in the dark and of coronal in the light for Algie). During these interleaved visual and tactile trials the animals made 11 and 15 errors, representing the same level of performance as achieved during learning within one modality.

The findings with these three chimpanzees provide no clear evidence of cross-modal transfer. Our other chimpanzees are to be tested exactly as those described in this report but only after they have received prolonged training for cross-modal recognition according to the procedure of Jarvis and Ettlinger (unpublished). The expectation is that they will then give evidence of good cross-modal transfer. Such a finding would, in agreement with other work¹, indicate that the chimpanzee can indeed attain cross-modal performance as a consequence of lengthy training. This is, however, also true for the monkey (Jarvis and Ettlinger, unpublished). Moreover, with the method of Cowey and Weiskrantz⁶ monkeys and chimpanzees¹¹ can additionally attain unidirectional cross-modal recognition without lengthy training. We therefore conclude that it remains to be shown whether important differences exist between the cross-modal capacities of apes and monkeys. The present experiment emphasises the importance of testing different species in truly comparable conditions.

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Table 1 Training sequence and performance for 3 chimpanzees on 5 cross-modal tasks

		Bertie	Algie	Blue
Adaptation				
Cylinder/Sphere	Tactile	720	420	120
Blue/Orange	Visual	190	290	300
First transfer test				
small/tall	Tactile	90	140	30
(size)	Visual	Reversal 270*(7)	90 (3)	Reversal 60 (7)
Second transfer test				
smooth/rough	Visual	0	0	90
(roughness)	Tactile	20 (7)	Reversal 190†(4)	0 (1)
Third transfer test				
L/T	Tactile	690	510	190
(size)	Visual	230 (5)	Reversal 120 (8)	80 (4)
Fourth transfer test				
pyramid/cone	Visual	380	50	120
(roughness)	Tactile	Reversal 40 (6)	220*(7)	Reversal 60 (9)
Fifth transfer test				
sagittal/coronal	Tactile	600 failed	0	0
(orientation)	Visual	—	Reversal 0 (4)	Reversal 40 (6)
Tactile and visual				
at random				
Errors in 80 trials		—	(11)	(15)

* Tests up to 27/30, within 300 trials.

† Tests to 35/40, within 300 trials.

Numbers give learning scores (up to 45/50, except on the fifth test when it was 160/200). Numbers in parentheses give errors on the first 10 trials in the new sense, except for lowest row where they represent errors in 80 trials of interleaved tactile and visual training with reward for reversed response. Scores for the second sense refer to non-reversed response, unless indicated.

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Novel surface polymer changes in development of *Myxococcus* spp.

THE Myxobacteriaceae are unique among prokaryotes in their development cycle. Vegetative cells are Gram-negative bacilli which undergo binary fission as in other prokaryotes; alternatively, the cells can aggregate on solid surfaces to form fruiting bodies in which the bacteria undergo conversion to myxospores. In *Myxococcus xanthus* and related species a simple fruiting body is formed in which spherical myxospores are surrounded by extracellular polysaccharide slime¹. In at least one strain of *M. xanthus*, synchronous myxospore formation can be obtained in liquid culture through induction with glycerol and other agents². The entire bacillus undergoes conversion to the myxospore form; there is no apparent loss of cell material such as is observed in the morphogenesis of Gram-positive bacilli to endospores at the expense of cell wall and surface material shed on emergence of the mature spore. The fate of the *Myxococcus* surface polymers is thus of particular interest.

M. xanthus has been the subject of numerous cytological studies^{3,4} which have indicated the formation of a thick extracellular coat together with a vesicular structure contained between this coat and the cytoplasmic membrane. The structural polymer, peptidoglycan, is present in cell walls of both bacilli and myxospores, although in a slightly modified form in the latter⁵. Both bacilli and myxospores are surrounded on solid media by extracellular polysaccharide which is of the same chemotype, and possibly identical, in bacillary cultures and in fruiting bodies⁶. We have now investigated the cell wall polysaccharides in bacilli and in myxospores, both those isolated from fruiting bodies and those obtained by glycerol induction. In particular, myxospores have been examined to determine whether they contained lipopolysaccharide.

Bacteria used were *M. xanthus* strain FB (Professor M. Dworkin, University of Minnesota) and a strain isolated from local soil (designated S). Strain FB underwent dispersed growth in liquid media and was inducible to myxospores by glycerol; strain S gave granular growth and was only poorly inducible to myxospores. The microanalytical procedures used have been described previously^{6,7}, except that acid hydrolysates were neutralised with Ba(OH)₂, separated into neutral and hexosamine fractions by preparative paper electrophoresis in pyridinium acetate buffer (pH 5.3) and analysed after elution. This procedure was

adopted because of the difficulty in obtaining uniform suspensions of myxospores and the use of hydrolysates of whole cells.

Lipopolysaccharide was present in the bacillary forms of the two strains and was extractable by the hot phenol procedure (65 °C, 5 min). It could then be deposited by ultracentrifugation at 100,000g for 4 h. Attempts to obtain lipopolysaccharide from myxospores of either strain were unsuccessful, although the bacilli had yielded 0.7–1.1% of their dry weight as sedimentable lipopolysaccharide. No carbohydrate-containing material was present in the phenol phase after extraction of myxospores as would be expected if the polymer had become more hydrophobic through loss of polysaccharide side chains⁸. As myxospores cannot be disintegrated by ultrasonic treatment or other readily available methods⁹ an alternative extraction procedure was required to obtain the polysaccharides known to be present¹⁰. The composition of these polysaccharides could be ascertained by analysis of hydrolysates of whole myxospores, as the only other monosaccharides known to be present are *N*-acetylglucosamine and *N*-acetylmuramic acid derived from peptidoglycan. The extraction procedure used by Freeman¹¹ to obtain side chain material from *Salmonella* lipopolysaccharides, 0.1 M acetic acid at 100 °C for 12 h, yielded carbohydrate-containing material from both bacilli and myxospores. From 95 mg freeze-dried myxospores of strain FB, 9 mg non-diffusible material was solubilised by the acetic acid treatment; the carbohydrate content was approximately 65%. Yields from strain S myxospores isolated from fruiting bodies were slightly lower. The carbohydrate composition of the lipopolysaccharides, acetic acid extracts and whole myxospores was compared by analysis and paper chromatography of hydrolysates (Table 1). Slight differences between the lipopolysaccharide composition found for strain FB and the results reported⁷ are almost certainly attributable to the improved accuracy of the analytical procedures when hydrolysates were freed from salts and other non-carbohydrate material by electrophoresis.

Chromatograms indicated that in strain FB, mannose present in the lipopolysaccharide had almost all disappeared from myxospores, while the ratio of glucose and galactosamine to glucosamine increased greatly in the myxospores. No appreciable differences were found between myxospores of this strain induced with glycerol and those obtained from fruiting bodies. Strain S lipopolysaccharide contained two sugars, mannose and galactose, which were almost entirely lost from myxospores; again there was an apparent increase in the glucose and galactosamine content. These observations were confirmed by analysis.

The two *Myxococcus* strains examined lack any of the more unusual sugars reported in the lipopolysaccharides of other Gram-negative bacteria—heptose, 3-*O*-methyl xylose and dideoxyhexoses are absent. Both, however, contain 2-keto-3-deoxyoctonic acid in their lipopolysaccharides⁷. Attempts to identify this compound in hydrolysates of

Table 1 Composition of polysaccharide preparations from *Myxococcus* strains FB and S

Strain	Preparation	Mannose	Glucose	Galactose	Glucosamine	Galactosamine	Ribose
FB	Lipopolysaccharide	15.3	31.2	0.3	18.9	29.0	5.1
	Lipid-free polysaccharide	21.3	35.0	0.3	16.1	23.7	3.5
	Freeman extract of bacteria	15.1	50.9	0.4	13.2	18.2	2.3
	Freeman extract of myxospores	0.2	67.7	0.2	5.2	24.9	0.6
	Myxospores*	0.9	63.1	0.2	6.8	26.7	0.7
	Myxospores†	0.7	69.0	0.4	5.4	23.5	0.5
S	Lipopolysaccharide	34.1	18.8	18.1	10.1	14.8	3.9
	Freeman extract of bacteria	30.6	18.9	14.9	15.4	15.4	3.5
	Myxospores†	7.3	51.2	5.8	9.9	23.4	2.4

*Glycerol induced; †Isolated from fruiting bodies.

Monosaccharide values are expressed as % total carbohydrate recovered after electrophoresis and elution.

myxospores were unsuccessful although it was identified on electrophoretic separation of the lipopolysaccharide hydrolysates. Although not conclusive in itself, this result, together with the loss of other monosaccharides, suggests that a hitherto unrecognised feature of the formation of myxospores from the bacillary forms of *M. xanthus* (and a phenomenon not previously reported for Gram-negative bacteria) is the complete loss from the wall of the polysaccharide portion of the lipopolysaccharide. The fate of the lipid A portion has not yet been ascertained. An analogy can thus be found to the loss of teichoic acid in bacterial endospore formation¹², except that the bacillary wall is not lost during myxospore development. Instead, there is an apparently selective rejection of the polysaccharide component of the lipopolysaccharide. It is not clear whether this is attributable to enzymatic removal or to ejection to make way for the new glucose- and galactosamine-containing polymer identified by Johnson and White¹⁰. Preliminary results suggest that replacement of lipopolysaccharide by a new polymer also occurs during myxospore formation in other fruiting species of Myxobacteria.

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Use of ionophore A23187 to measure cytoplasmic Ca buffering and activation of the Ca pump by internal Ca

DURING the formation of the mature mammalian erythrocyte, the reticulocyte sheds its nucleus, endoplasmic reticulum and mitochondria leaving a featureless cytoplasm with a high haemoglobin content surrounded by a plasma membrane. All Ca-accumulating organelles are lost and the mature red cell is maintained virtually Ca-free throughout its life¹ by the low Ca permeability of its membrane² perhaps aided by a powerful Ca-extrusion pump³. Most of our knowledge of Ca transport in red cells comes from studies with resealed ghosts, but in this preparation the results are often affected by alterations to the membrane and metabolism of the cell²⁻⁴. The main difficulty of using intact fresh cells in physiological conditions has been the impossibility of increasing their Ca content in a controlled way and of assessing the fraction of Ca which is ionised. Using a divalent-cation ionophore, A23187 (ref. 5), however, we have overcome these difficulties and found that cytoplasmic Ca buffering occurs as if the cell had a single large-capacity, low-affinity Ca buffer and that there are two Ca-translocating sites of equal affinity at the internal surface of the Ca pump.

Preliminary experiments showed that in the red cell the ionophore selectively increases the Ca and Mg fluxes without affecting directly the Na and K fluxes, in agreement with its action in other systems⁵. The incorporation of the ionophore into the red cell membrane induces a large Ca leak. In these conditions, the rate of change of the total Ca concentration in the cell ($\mu\text{mol per l cells per h}$) is given by

$$d\text{Ca}_t^T/dt = P(r^2\text{Ca}_0 - \alpha\text{Ca}_t^T) - \phi \quad (1)$$

where P represents the ionophore-induced leak permeability

for Ca (h^{-1}); r^2 is the Donnan distribution ratio for divalent ions at equilibrium and is related to the membrane potential (V_m) by the expression $r^2 = \exp(-2FV_m/RT)$; α is the fraction of ionised Ca inside the cell; Ca_0 is the concentration of ionised Ca in the medium and ϕ is the Ca extrusion rate through the Ca pump ($\mu\text{mol per l cells per h}$). The maintenance of steady states for Ca_t^T (see Fig. 1) suggests that the Ca pump remains saturated with ATP for the duration of our experiments. Following Schatzmann³ the pump-flux term can therefore be expressed as

$$\phi = \phi_m[\alpha\text{Ca}_t^T/(\alpha\text{Ca}_t^T + K)]$$

where ϕ_m represents the maximum Ca pumping rate and K is the Ca dissociation constant at the internal surface of the pump.

Red cells from fresh blood or from 3-4-d-old bank blood were washed 3 times with about ten volumes of ice-cold solution containing NaCl, 75 mM; KCl, 75 mM; Tris-Cl (pH 7.5 at 37 °C), 10 mM and Tris-EGTA (1,2-Di(2-aminoethoxy) ethane-NNN'-N'-tetraacetic acid), 0.1 mM and 4 times more with the same solution without Tris-EGTA. After the washes the cells were packed at 2,000g for 5 min and the supernatant disposed of. At $t = 0$, 0.25 ml of packed cells were added to 2 ml of a medium at 37 °C stirred magnetically and containing: NaCl, 75 mM; KCl, 75 mM; Tris-HCl (pH 7.5 at 37 °C), 10 mM; MgCl_2 , 1-2.33 mM; Inosine, 10 mM, 5 μCi of ^{45}Ca and varying amounts of CaCl_2 . This particular K concentration was used to minimise the effect which a Ca-induced increase in K permeability⁶ might have had on the membrane potential and, therefore, on r . Samples were withdrawn from this cell suspension at suitable time intervals and processed as detailed in the legend of Fig. 1.

Figure 1 shows two representative time curves at initial concentrations of Ca_0 of 93.2 and 1,000 μM . In this experiment, at 93.2 μM Ca_0 and the lower concentrations, the uptake of Ca at 30 s went through a maximum considerably larger than the steady-state value, whereas at higher Ca_0 the kinetic pattern followed was that represented by the 1,000- μM curve. The transient peak was never observed in ATP-depleted cells⁷ or at higher external Ca concentrations in fed cells. Its dependence on the metabolic state of the cell suggests an initial inactivity of the Ca pump. Successive additions of ionophore to fed cells in the presence of lower Ca concentrations, however, always elicits a transient peak response. A possible explanation of these peaks is that initially only a fraction of the cells takes up all the ionophore, thus behaving like the high-ionophore cells in Fig. 2. As the ionophore becomes more homogeneously distributed, Ca is pumped out from these cells and a final steady state with a lower average Ca content is achieved. If ^{45}Ca is added during steady state to cells containing enough Ca to saturate the pump, the final ^{45}Ca distribution is the same as in controls with ^{45}Ca present from the beginning but is approached exponentially without any maxima. In depleted cells, the steady-state distribution of Ca was unaffected by further additions of ionophore, but in fed cells this was true only for very high initial ionophore concentrations (above 100 $\mu\text{mol per l cells}$).

The use of α in equation (1) to describe the buffering of Ca by the red cell involves a number of simplifying assumptions which require consideration. In the general case for n intracellular buffers the relation between total and free Ca is described by

$$\text{Ca}_t^T = \text{Ca}_t^{2+}[(V_w/V_c) + \sum_{i=1}^n \{B_i/(K_i + \text{Ca}_t^{2+})\}] \quad (2)$$

where V_w is the volume of cell water per litre of cells (V_c); B_i , the total concentration of the i th buffer, and K_i the dissociation constant between Ca and this buffer.

In ATP-depleted cells⁷, the ionophore induced the red cell

membrane to act like a "dialysis bag" for Ca. The equilibrium distribution of Ca as a function of external Ca showed that Ca buffering can be satisfactorily described assuming the cells have a single large-capacity low-affinity Ca buffer in agreement with earlier results obtained with cell lysates³. In this case, equation (2) becomes

$$Ca_i^T = Ca_i^{2+}[(V_w/V_c) + B/(K_B + Ca_i^{2+})]$$

If $Ca_i^{2+} \ll K_B$ then

$$Ca_i^T = Ca_i^{2+}[(V_w/V_c) + (B/K_B)]$$

and

$$\alpha = [(V_w/V_c) + (B/K_B)]^{-1}$$

The use of V_w/V_c already allows for the fact that while Ca_i^T is expressed per litre of cells, Ca_i^{2+} represents the actual concentration of ionised Ca per litre of cell water.

In fresh cells, the buffering capacity was estimated from the slope of the curve relating the steady-state internal Ca concentration (Ca_i^T) with the external free Ca concentration (Ca_0)

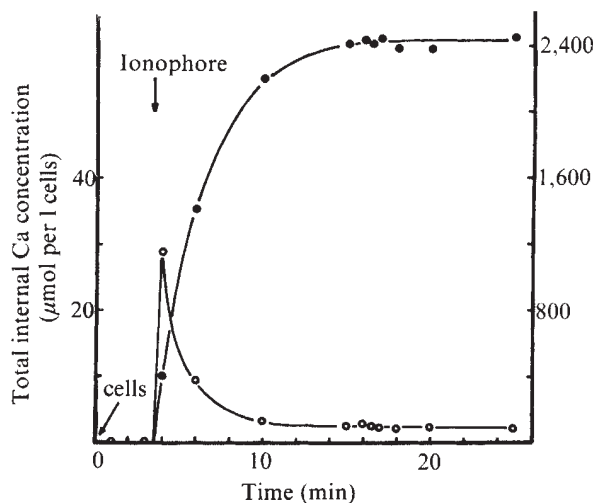


Fig. 1 Ca content of cells as a function of time at two different external Ca concentrations. At the times indicated 0.1-ml samples of the cell suspension were placed in 1.5-ml Eppendorf centrifuge tubes containing 0.4 ml *n*-butyl-phthalate (BDH, density 1.042–1.045) and 0.9 ml of the initial washing medium but with 5 mM Tris-EGTA, all at 0–1 °C. Each sample was centrifuged immediately for 10 s at 12,000g in an Eppendorf centrifuge model 3200. The cells formed a compact pellet at the bottom of the oil layer and the aqueous supernatant remained on top. The whole procedure from sampling to cell separation took about 20 s. The supernatant, including the oil, was sucked off and the walls of the tube cleaned and dried with cotton swabs. The cells were lysed in 0.5 ml of distilled water, the proteins precipitated with 5% TCA and aliquots used for counting. Samples for total activity and haemoglobin were taken at the end of each run. The extracellular activity trapped in the cellular pellet was estimated using ⁴⁵Ca in the presence of a large excess of Tris-EGTA. The cells were added at $t = 0$ and the ionophore was added after the second sample, at $t = 3.5$ min in 10 μl of absolute ethanol to give a final concentration of 10 μmol per l cells (assuming complete partitioning of the ionophore into the cells). Ordinates: Ca content of cells (in μmol per l original cell volume). Note the fortyfold difference in scale between left and right ordinates corresponding to the curves at low and high external Ca concentration, respectively. ○, Initial external Ca concentration, 93.2 μM, final steady state concentration, 90.7 μM; ●, initial external Ca concentration: 1,000 μM, final steady state concentration, 740 μM. Haematocrit, 8.9%. The increased frequency of sampling during the steady state is due to the fact that ⁴²K was added at $t = 15.5$ min to measure simultaneously the K influx associated with each particular steady Ca level. (The results on the relationship between potassium permeability and the internal free Ca concentration in the intact fresh red cell will be published elsewhere.) The curves represent the kinetic patterns obtained with eleven concentrations of external Ca tested (see text).

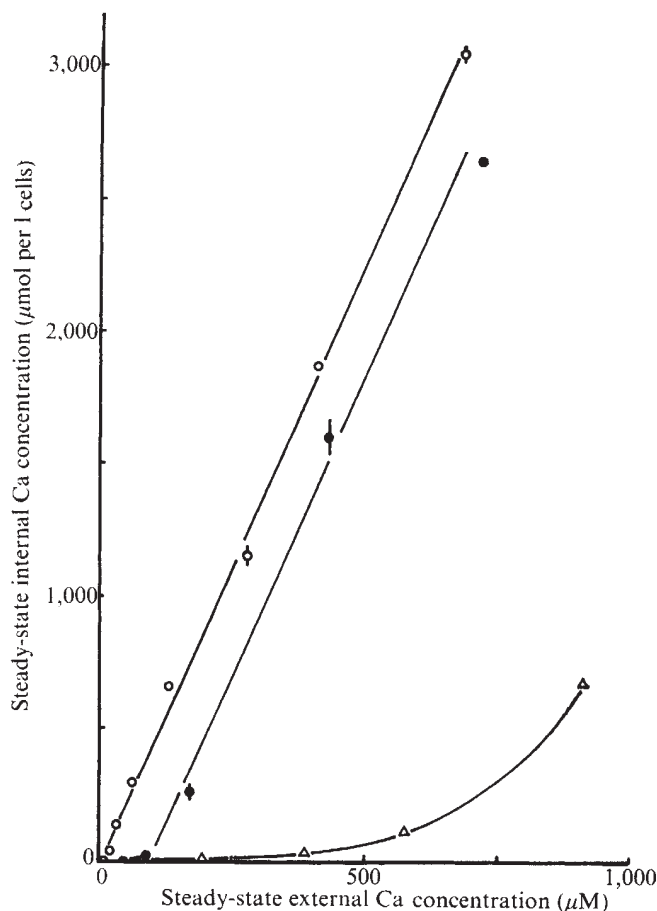


Fig. 2 Total Ca content of red cells as a function of external Ca concentration during steady state. In this experiment we used three ionophore concentrations: ○, 100 μmol per l cells, $I = 100$; ●, 25 μmol per l cells, $I = 25$; and △, 6.3 μmol per l cells, $I = 6.3$. Assuming complete partitioning of the ionophore into the cells, the highest concentration corresponds to about 6×10^6 molecules per cell. If it is all partitioned into the red cell membrane, at the highest concentration there would be roughly one molecule of ionophore per $2,600 \text{ Å}^2$ of membrane which, with a molecular weight of 523 (ref. 5), will cover probably less than 0.5% of the cell surface. The value of α in this experiment was 0.43 and it was unaffected by the concentration of ionophore used. It was assumed that $r^2 = 2$ which corresponds to a steady-state membrane potential of about 10 mV.

when the Ca pump became saturated (see Fig. 2). When $dCa_i^T/dt = 0$ (steady state) and $\phi = \phi_m$ equation (1) becomes

$$Ca_i^T = (r^2/\alpha)Ca_0 - (\phi_m/\alpha P) \quad (3)$$

This relation between Ca_i^T and Ca_0 was shown to be valid over a wide range of ionophore and Ca concentrations (see Fig. 2). The value of α varied from 0.3 to 0.5 among cells from different donors indicating that the fraction of ionised Ca represents between 30% and 50% of the total intracellular Ca concentration.

We then calculated the value of the dissociation constant for Ca at the internal pump site. At relatively low external Ca concentrations, the Ca pump is capable of maintaining large Ca gradients across the membrane during steady state (see Fig. 1), and in these conditions $dCa_i^T/dt = 0$ and $r^2Ca_0 \gg \alpha Ca_i^T$. Equation (1) can be rearranged to give

$$Ca_0 = (\phi_m/r^2P)[\alpha Ca_i^T/(K + \alpha Ca_i^T)] \quad (4)$$

At similar external concentrations, equation (4) can be used to compare the measured value of αCa_i^T from different experiments and at different ionophore concentrations. If x and y represent the ionised Ca concentration (αCa_i^T) obtained in two different

experiments, or at two different ionophore concentrations, then, equation (4) becomes

$$Ca_0 = C_1[x/(K+x)] = C_2[y/(K+y)] \quad (5)$$

where C_1 and C_2 represent the constant factor (ϕ_m/r^2P) for the two conditions that are being compared. Equation (5) can be rearranged to give

$$1/x = (C_1/C_2)(1/y) + [(C_1/C_2) - 1]/K \quad (6)$$

This equation predicts a linear relationship from which the value of K can be calculated. Figure 3 illustrates such a calculation and confirms the linear relation predicted by equation (6). The values of K obtained by this method varied between 0.73 and 1.02 μM . Note that K calculated in this way is independent of the stoichiometry of transport, since it remains invariant if equation (5) were

$$C_1[x/(K+x)]^n = C_2[y/(K+y)]^n$$

provided that the different translocating sites have similar affinities.

This value of K was then used to plot $(r^2Ca_0 - \alpha Ca_i^T)$ as a function of the pump saturation ratio $[\alpha Ca_i^T/(K + \alpha Ca_i^T)]$ in all the conditions tested (Fig. 4). The linear relationship predicted by equation (1) was only confirmed when we used a stoichiometry of two translocating sites (see Fig. 4).

The slope of the curve in Fig. 4 (slope = ϕ_m/P , equation (1)) could be used to estimate ϕ_m if P were known. We can only obtain a rough estimate of P from the ratio between the Ca uptake at 30 s and r^2Ca_0 . This gives values of P between 7 and 20 h^{-1} which correspond to values of ϕ_m of about 7 to 12 mmol per l cells per h. This is considerably lower than the value expected at 37 °C from Schatzmann's data in resealed ghosts³.

This study introduces a general method by which the Ca concentration of the intact red cell can be varied in a controlled manner and its ionised fraction assessed. The method could also be applied to studies with Mg and opens new possibilities for the investigation of all the mechanisms involving divalent cations in the red cell. We report here a first application of this method. The binding of Ca at the internal Ca-pump surface can be described as taking place at two sites with similar dissociation constants around 1 μM . This value closely agrees with a half-saturation concentration of 4 μM or less for Ca translocation reported by Schatzmann³. Schatzmann, however, suggested a stoichiometry of one Ca per ATP hydrolysed and although we have not yet measured the hydrolysis of

Fig. 3 The relationship between the reciprocals of the steady-state intracellular concentrations of Ca at similar low external Ca levels of the two lower ionophore curves of Fig. 2. The continuous line corresponds to the equation

$$1/x = -0.179 + 0.577(1/y); K = 1.02$$

and is interpreted according to equation (6) in the text.

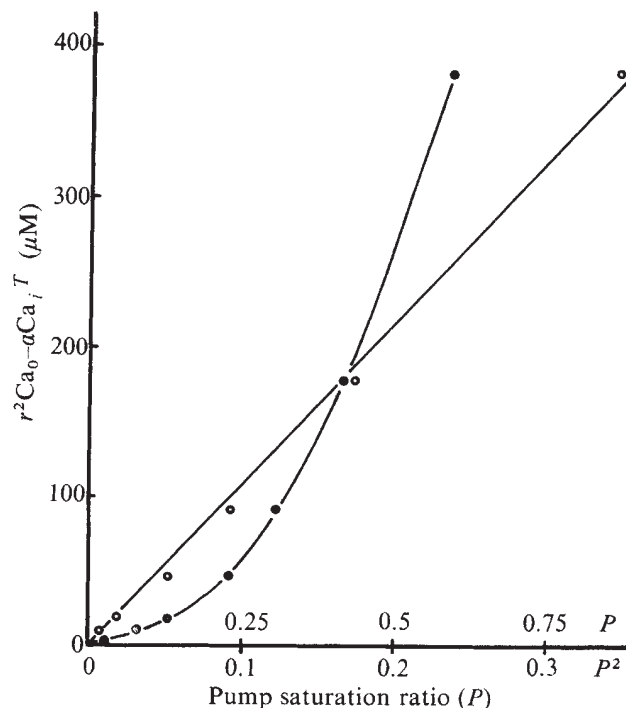
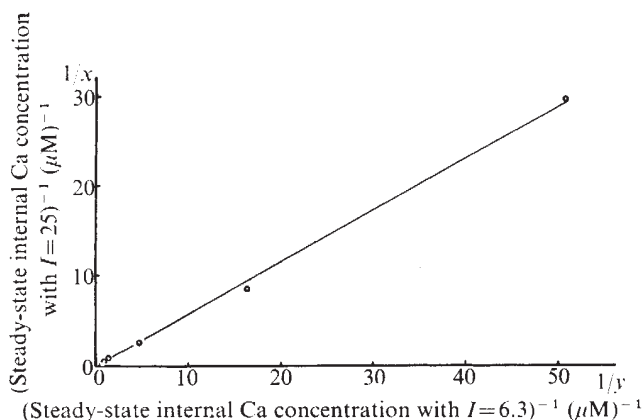


Fig. 4 The relationship between the steady-state value of $(r^2Ca_0 - \alpha Ca_i^T)$ and the pump saturation ratio

$$P = [\alpha Ca_i^T / (K + \alpha Ca_i^T)]^n$$

according to equation (1). The value of K used was 1.02 μM as obtained from Fig. 3. It can be seen that the straight line relationship predicted by equation (1) is obtained only if the square of the pump saturation ratio is used. This curve, corresponds to the curve obtained with the lowest ionophore concentration of Fig. 2. ●, $n = 1$; ○, $n = 2$.

ATP associated with Ca extrusion, our results firmly support a translocating stoichiometry of two Ca ions per pump cycle. This suggests a closer similarity with the sarcoplasmic reticulum pump⁷.

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L-leucine, a specific inhibitor of a rare human placental alkaline phosphatase phenotype

FISHMAN *et al.* reported that human placental alkaline phosphatase (Regan isoenzyme) was found in the serum and tumour tissue of a patient with lung cancer¹. Two electrophoretically different variant forms of Regan alkaline phosphatase with a greater sensitivity to inhibition by L-leucine have since been described: the 'Nagao isoenzyme'²,

and a hepatoma-specific alkaline phosphatase³⁻⁵. Inglis *et al.*⁶ reported that the L-leucine-sensitive Nagao isoenzyme found in some cancer patients closely resembled the 'D variant' of human placental alkaline phosphatase, a rare phenotype defined electrophoretically on starch gel which is found in less than 1% of the general population of pregnancies⁷. An example was reported of a pregnancy serum D-phenotype enzyme presumably of placental origin, confirmed by electrophoresis established as L-leucine-sensitive⁶. Most interesting was the occurrence of the variant at high frequency in the ovarian cancer patients surveyed^{6,8}. To extend the observations of Inglis *et al.*, screening for the phosphatases in placentae was carried out, both to establish the frequency of the L-leucine-sensitive placental phenotypes (which should match the frequency of the electrophoretically defined D variant phenotypes) and to provide enough enzyme to enable purification and characterisation. Supply of cancer tissue is generally restrictive for this purpose.

Using a sample of ovarian cancer ascitic fluid containing the L-leucine-sensitive variant⁶ as reference standard, we found nine placentae out of a total of 2,323 placentae screened (frequency 0.0039) which closely resembled the cancer patient enzyme in sensitivity to L-leucine. Normal placentae were obtained from St Margaret's Hospital, Dorchester, immediately after delivery and stored at -20 °C. Trophoblast tissue (5-15 g) was excised from each frozen placenta using a bone scalpel and homogenised with two volumes 0.9% saline per g tissue. The homogenate was centrifuged at 6,000g for 10 min and the supernatant diluted 1:100 with 0.01 M Tris-HCl-buffered saline, pH 8.3.

Alkaline phosphatase assay was carried out as described previously⁹ using 18 mM phenyl phosphate as a substrate in 0.05 M sodium carbonate-bicarbonate buffer, pH 9.8, containing 10 mM MgCl₂. To determine the optimum concentration of L-leucine, a partially purified enzyme preparation from an ovarian cancer patient was assayed for L-leucine inhibition. It was found to be 43% inhibited at a concentration of 0.5 mM L-leucine, using 0.5 mM D-leucine as a reference, whereas a non-variant placental enzyme was inhibited 8%. L-leucine (0.5 mM) was therefore used for screening, using the same concentration of D-leucine in the control sample. Each diluted sample (100 µl aliquots) was assayed for 30 min at 37 °C in 0.5 mM D- and L-leucine-containing substrate solutions. Inhibition of the 2,314 normal placentae was $7.8 \pm 4.3\%$, demonstrating that the common phenotypes of placental alkaline phosphatase⁷ are not inhibited by L-leucine. Only the nine variants were outside the normal range (Table 1). Average inhibition profiles for the enzymes from nine individual variants and five normal placentae are shown as a function of L-leucine concentration. At all L-leucine concentrations tested, the variant enzymes were different from normal, this difference reaching a maximum of 40% at 0.5-2.0 mM L-leucine.

If the variant placentae are heterozygotes⁷ consisting of 50% common phenotypic enzyme and 50% L-leucine-

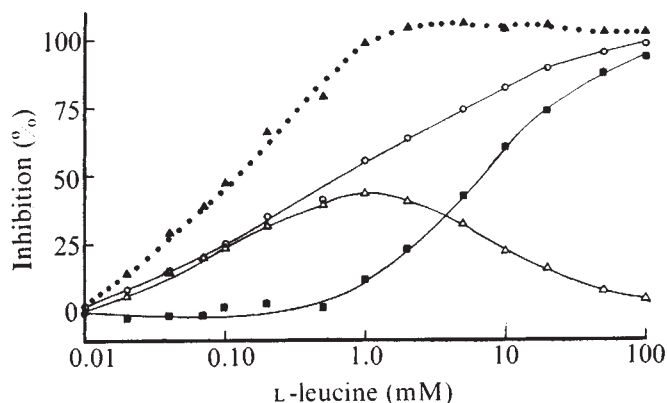


Fig. 1 Average inhibition profiles in increasing concentrations of L-leucine: ○, variant enzyme (cv); ■, normal enzyme (cc); △, difference between inhibition of variant and normal enzymes (cv-cc); ▲, difference between twice the inhibition of heterozygous variant enzyme and normal enzyme (2cv-cc).

sensitive variant enzyme, it would be reasonable to estimate the inhibition of the L-leucine-sensitive moiety alone (vv) from the expression:

$$2cv - cc = vv$$

where cv represents the inhibition of the heterozygote made up of the common and variant phenotypes, cc the inhibition of placentae containing only the common phenotypes, and vv the inhibition expected for a homozygous variant enzyme. The approximation used here makes two assumptions: that the turnover number of the variant and common phenotypic enzymes for 18 mM phenyl phosphate are identical, and that there is no cooperativity in the inhibition of normal and variant enzyme in the heterozygote.

The result of this calculation for a full range of L-leucine concentrations is shown in Fig. 1 as the dotted line. The curve for vv reaches a level of 100% inhibition at about 1 mM L-leucine (the concentration at which the difference between the common and variant enzymes is maximal), and remains constant at 100-105% between 1 and 100 mM L-leucine. By comparison with the common placental profile cc, which reaches a value of 50% inhibition at an L-leucine concentration of 7.3 mM, the vv profile shows an inhibition of 50% at about 0.12 mM, or a 60 times lower concentration of L-leucine. This difference in the concentration of L-leucine which inhibits 50% is a convenient reference value for comparison of the common cc and variant vv inhibitions. Similarly, the difference between the inhibition of the heterozygous variant and common placental enzymes at the L-leucine concentration at which this difference is maximal (that is, 0.5-2.0 mM L-leucine) can also be regarded as a basis for comparison of the variant enzymes of placentae and cancer patients. Such a comparison is made in Table 1—reference cancer fluid and nine placental enzymes found.

Since the cancer patient enzyme was inhibited to the same extent as the heterozygote placental variants in 0.5 mM L-leucine, the leucine-sensitive Nagao isoenzyme in the sera and the fluid of cancer patients can be assumed to reflect the expression of both common and variant genes, and therefore to be a heterozygote. A similar degree of L-leucine inhibition has been reported for the Nagao isoenzyme² and for the electrophoretically fast-moving hepatoma-specific alkaline phosphatase^{4,5}.

In connection with the greater L-leucine sensitivity of the variant, note that the stereospecific inhibition of mammalian alkaline phosphatase by L-amino acids^{13,14} is not reported for any prokaryotic alkaline phosphatases. The variant enzyme described in this work should enable a more detailed study of this mode of inhibition; much

Table 1 Inhibition of extracts of nine variant placentae by 0.5 mM L-leucine, using 0.5 mM D-leucine as control compared with cancer ascites fluid

	% Inhibition ($\pm$ s.d.)
Cancer ascites fluid	46.2 $\pm$ 0.8
Variant 1	45.8 $\pm$ 1.6
Variant 2	45.6 $\pm$ 1.0
Variant 3	43.5 $\pm$ 0.7
Variant 4	46.2 $\pm$ 0.7
Variant 5	48.6 $\pm$ 1.6
Variant 6	47.0 $\pm$ 1.0
Variant 7	44.5 $\pm$ 1.3
Variant 8	48.8 $\pm$ 1.0
Variant 9	48.7 $\pm$ 1.2

Assays were run in pentuplicate. Cancer ascites fluid provided by Dr L. L. Stolbach contained exclusively the variant enzyme (Nagao).

as the availability of mutant forms of bacterial enzymes, which are less feedback-inhibited by amino acids, has facilitated the study of feedback inhibition¹⁵.

The relationship of the variant enzyme to cancer is implicit in the similarity of the leucine sensitivity of the cancer patient enzyme with that of the placental variant, and in the similarity in immunoreactivity of this enzyme⁹. These two observations alone could not, however, be regarded as proof that the two enzymes represent the same genomic origin, or that the enzyme may mask a predisposition to certain cancers. Detailed structural and kinetic studies are being carried out to determine whether the variant and ascites enzymes are in fact identical in all respects. Additionally, epidemiological studies on the occurrence of various types of cancer in the families of children bearing the variant enzyme gene will be necessary.

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Late acquisition of a germ line antibody specificity

THE humoral immune response to most antigenic determinants is characterised by a highly heterogeneous population of antibody molecules derived from a large array of precursor cell clonotypes¹⁻³. The response to certain antigenic determinants, however, is relatively restricted and may be dominated by a single homogeneous antibody species. In inbred strains of mice, such dominant antibody specificities are present in all individuals of a strain⁴⁻⁹, are characterised by identical idiotypic determinants⁴⁻⁸, and are linked to heavy chain allotype markers⁷⁻¹⁰. Thus, these clonotypes are presumed to be a direct reflection of genetic information present in the germ line. A good example of an antibody specificity considered to be 'germ line' is the major clonotype responsive to phosphorylcholine in BALB/c mice, which is identical in its combining region to the TEPC 15 and S107 plasmacytoma proteins⁴⁻⁵. Representatives of the cell clone producing this phosphorylcholine-specific antibody have been found in high frequency (19 ± 6 per 10^6 bone marrow-derived antibody-forming cell precursors (B cells)) in both conventionally reared and germ-free BALB/c mice¹¹.

'Germ line' specificities can be enumerated by analysing the specificity repertoire of neonatal mice^{3,12,13}. Since few

B cells are present during the first few days after birth, the neonatal repertoire may be thought to be quite restricted. Examination of the available clonotypes specific for the haptens dinitrophenol (DNP) and trinitrophenol (TNP) immediately after parturition revealed at least six clonotypes, three specific for DNP and three for TNP, present repeatedly in different individuals¹³. These predominant clonotypes can be represented by many (>100) cells. Other clonotypes specific for DNP or TNP occur only sporadically in the early neonatal B-cell population. By the seventh day after birth, however, such sporadic clonotypes represent most of the precursor pool, and presumably represent clonotypes expressed later in development than the early predominant ones¹³. It is conceivable that the all or none nature of the neonatal response to antigens like fluorescein^{3,13} and the temporal hierarchy observed in the appearance of foetal and neonatal responses^{14,15} may be accounted for by sporadic or late occurring clonotypes.

Since precursor cells representing the early predominant clonotypes occur with great regularity among individuals, and are present in large numbers at birth, they presumably were initiated early in foetal life and thus may be close reflections of the germ line genetic information for antibody specificity¹³. It should be interesting, therefore, to ascertain whether the dominant clonotypes observed in adult responses, for example, the TEPC 15 clonotype specific for phosphorylcholine, are expressed as neonatal predominant clonotypes, and thus fulfil another criterion for 'germ line' representation. In this report, the frequency of phosphorylcholine-specific B cells is quantitated in neonatal BALB/c mice. The results indicate that phosphorylcholine-specific clonal precursor cells, even those of the dominant TEPC 15 clonotype, appear quite late in neonatal development.

Spleen cells from BALB/c mice at various ages from birth to adulthood were transferred intravenously to lethally irradiated (1,300–1,600 r.) adult BALB/c mice immunised 2–3 months previously with 0.1 mg of *Limulus polyphemus* haemocyanin (Hy)². Sixteen hours after transfer, spleen fragment cultures were prepared from recipients as before², and cultures were stimulated *in vitro* for 3–4 d with either 1×10^6 M DNP-Hy or 5×10^7 M 3-(p-azo-phenylphosphorylcholine) - N - acetyl - L - tyrosylglycylglycine - haemocyanin (PPC-TGG-Hy), an antigen shown to maximise carrier recognition in anti-phosphorylcholine responses⁶. Culture fluids collected from day 9 to day 13 after culture initiation were analysed for the anti-phosphorylcholine or anti-DNP antibody by a sensitive radioimmunoassay. The binding of antibody to the antigen coupled to bromoacetyl cellulose immunoadsorbent was detected with ¹²⁵I-labelled rabbit anti-mouse Fab antibody^{2,6}. Previous studies have demonstrated the donor origin of the antibody-producing cells¹⁶, the monoclonal nature of the antibody obtained^{2,12,16}, the efficiency of detecting clonal precursors in this system (3.9%) (ref. 16 and unpublished results of A. Pickard and N.R.K.), and the efficiency of such stimulation for the analysis of neonatal B cells^{3,12,13,16}. Antibody with the TEPC 15 idiotype was detected in an inhibition solid-

Table 1 Number of phosphorylcholine-specific foci and percentage of those foci positive for the TEPC 15 idiotype at various ages

Age (d)	Total No. of neonates	Total No. of cells analysed	No. of foci	% Foci with TEPC 15 idiotype
0-2	53	148×10^6	0	—
3-5	15	66×10^6	0	—
6-7	34	628×10^6	26	66
8-9	22	320×10^6	13	77
14	26	823×10^6	56	72
21	6	231×10^6	24	57
28	3	199×10^6	25	72

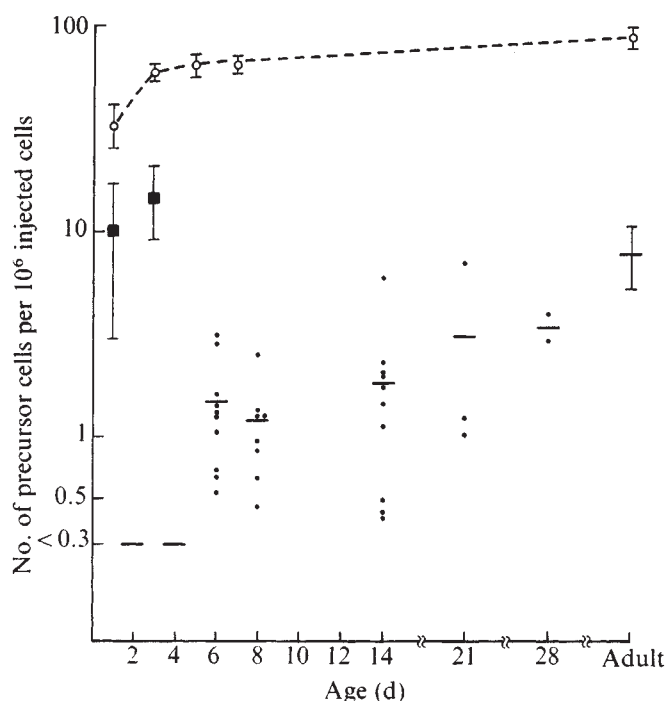


Fig. 1 The frequency of clonal precursor cells specific for phosphorylcholine or DNP at various ages. Two to 25×10^6 donor spleen cells from conventionally reared mice were transferred into an individual recipient, and the recipient spleen fragments were stimulated with PPC-TGG-Hy or DNP-Hy. Each small circle represents the phosphorylcholine-specific precursor cell frequency derived from one to ten donor spleens injected into two to eight Hy-primed recipients; the bars indicate the average frequency at each age. The adult precursor cell frequency for phosphorylcholine is derived from data published before¹¹. Since no phosphorylcholine-specific foci were detected in 2- and 4-d-old donors, the bars at those ages denote the maximum precursor frequency if one focus had been detected. The open circles represent the average DNP-specific precursor cell frequency at various ages; the data have been published for neonates¹³ and adults^{1, 16}. The closed boxes indicate the average frequency and standard deviation of single DNP-specific predominant clonotypes, assuming that each of the three predominant clonotypes is represented equally in the early neonatal period. All the data are represented as the frequency of clonal precursor cells in 10^6 spleen cells, using a cloning efficiency of 3.9% as the correction factor¹⁶.

phase radioimmunoassay using anti-TEPC 15 sera raised in A/He mice⁶.

Table 1 shows that of the 53 neonates examined from birth to 2 d of age and the 15 neonates analysed from 3 to 5 d of age, no foci producing anti-phosphorylcholine antibody were detected. The first phosphorylcholine-specific clonotypes arise approximately 1 week after birth, and most of these clones are of the TEPC 15 idiotype, as is the case in the BALB/c adult⁶. Figure 1 plots the frequency of DNP and phosphorylcholine-specific precursor cells in adults and neonates during the first several weeks of life. As reported previously, DNP-specific cells are present in high frequency at birth and remain relatively constant into adulthood^{3, 12}. Conversely, phosphorylcholine-specific precursor cells do not appear at all until day 6–7 after birth and remain in relatively low frequency for several weeks. Although, as Fig. 1 demonstrates, both conventionally reared neonates and adults show wide variations in phosphorylcholine-specific precursor frequency, the average neonatal frequency at 1 week old is eight times lower than the average adult precursor cell frequency.

The DNP-specific precursors present in the spleen during the first 5–6 d after birth are largely representative of only three predominant clonotypes¹³. A DNP-specific B cell represents one of 3,000 total B cells; therefore, the frequency of any one of the predominant clonotypes is one in

10^4 B cells. This is the same frequency observed for the three TNP-specific predominant clonotypes, which have been shown to be distinct from those specific for DNP by isoelectric focusing¹³. Thus while the frequency of each of the six known neonatal predominant clonotypes is one in 10^4 B cells, the data presented here indicate that early in development the TEPC 15 clonotype is 100 times less frequent than clonotypes presumably representing the earliest expression of the germ line genetic information for antibody specificities.

An analysis of 26 germ-free adults from various sources shows a frequency similar to conventionally reared adults, so that it is unlikely that antigen contact plays a significant role in either the generation of precursors with the TEPC 15 idiotype or the maintenance in high numbers in adult mice¹¹. Preliminary studies using germ-free neonates 5 and 8 d old demonstrate that the kinetics of appearance of phosphorylcholine-specific precursors in germ-free neonates is, in general, similar to that seen in conventionally reared mice (our unpublished results). Although neither maternal influences nor the presence of antigen can be ruled out specifically as the reason for the late acquisition of the TEPC 15 clonotypes during the neonatal period, the preliminary data with the germ-free neonates suggests that this is not the case.

Since the TEPC 15 clonotype is a prototype of a 'germ line' specificity, its late appearance during ontogeny may have significant implications for the mechanism by which the B-cell repertoire is acquired. This report and others have shown that the generation of the repertoire seems to be antigen independent^{3, 12, 13, 16}, and the findings reported here indicate that the expression of even ubiquitous clonotypes may be relatively late events in neonatal development.

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Classical pathway activation of complement system by IgA anti-C3 antibody

ANTIBODIES which react with native C3 are present in high titre in adult¹ and neonatal saliva². Although they belong to the IgA class they are macromolecules with a sedimentation coefficient of greater than 19S. They are also unusual in that their binding to C3 is dependent on the presence of

Table 1 C3 conversion induced by neonatal anti-C3 antibody

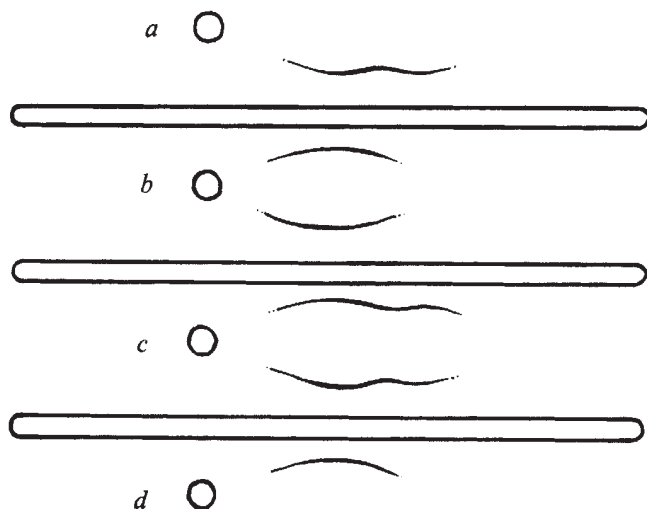
	EDTA plasma (%)	EGTA plasma (%)	Serum (%)	C2 Deficient serum (%)
IgG (50 µg)	< 5	< 5	38	5
Zymozan	< 5	31	36	23
Anti-C3 1:5	< 5	5	31	< 5
Anti-C3 1:10			27	7
Anti-C3 1:20			17	< 5

calcium ions and they can be dissociated from C3 by 0.01 M EDTA^{1,2}. Our preliminary studies on the properties of this antibody had shown that it could activate the complement system and in this paper we report investigations to determine whether this proceeds by way of the classical or alternative pathway.

Activation of the complement system was assessed by measuring the conversion of C3 occurring in normal human serum after adding anti-C3 antibody, the amount of conversion being calculated by planimetry³. Dilutions of purified antibody² were added to equal volumes of fresh human serum, EDTA plasma, EGTA plasma and C2-deficient human serum, and then incubated at 37 °C for 45 min. Heat-aggregated IgG and zymozan were included as positive controls. The results obtained are summarised in Table 1. Both the aggregated IgG and the zymozan produced C3 conversion in normal serum. The aggregated IgG did not convert C3 in EGTA plasma or C2 deficient human serum whereas zymozan produced C3 conversion in both sera. Conversion of C3 occurred when purified anti-C3 antibody was added to human serum and the amount of conversion was proportional to the amount of antibody added. No conversion of C3 occurred when antibody was added to C2-deficient human serum or EGTA plasma. C3 conversion in normal guinea pig serum and C4-deficient guinea pig serum was assessed qualitatively after immunoelectrophoresis in 1% agar, diluted in barbitone buffer pH 8.6 containing 0.01 M EDTA. The antibody, although capable of converting C3 in normal guinea pig serum, failed to convert C3 in C4 deficient serum (Fig. 1).

The ability of the antibody to activate C1 directly was

Fig. 1 Results of immunoelectrophoresis showing conversion of C3 in normal guinea pig serum (GPS) but no conversion in C4 deficient guinea pig serum (C4D). *a*, C4D and zymozan; *b*, C4D and anti-C3 antibody; *c*, GPS and anti-C3 antibody; *d*, GPS, EDTA to 0.01 M and anti-C3 antibody. Trough contains goat anti guinea pig C3 (Nordic Diagnostics).



then examined in serum from a patient with hereditary angioneurotic oedema which contained only 10% of the normal concentration of C1 inhibitor. Anti-C3 antibody (100 µl) was added to 800 µl of complement buffer, 100 µl of serum and 50 µl of 1 M acetyl tyrosine ethyl ester and then incubated with constant stirring at 37 °C. C1 was measured enzymatically by the hydrolysis of the ester⁴. When antibody was excluded from the mixture very little spontaneous activation of C1 took place, but the addition of purified antibody or heat aggregated IgG resulted in rapid activation of C1.

The observations that anti-C3 antibody in combination with antigen can activate C1 directly and that it fails to convert C3 in C2 and C4 deficient serum indicates that complement activation requires the early components C1, C4 and C2. In man, previous reports have shown that complexes or aggregates of IgA activate the complement system via the alternative pathway⁵. Our studies on the salivary anti-C3 antibodies show for the first time that IgA antibody can also activate the complement system via the classical pathway. This pattern of activation may account for the observations that the haemolytic titres of C3 and C4 are low and that inactivated forms of these two proteins are present in colostrum⁶, this secretion being another source of the anti-C3 antibody in humans⁷. Since these antibodies are macromolecular in size and have a number of unusual properties our findings may not be applicable to other IgA antibodies.

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Antibodies against homogeneous epoxide hydratase provide evidence for a single enzyme hydrating styrene oxide and benz(a)pyrene 4,5-oxide

THE microsomal enzyme epoxide hydratase (EC 4.2.1.63) is potentially important in the inactivation of metabolically produced epoxides which may be responsible for the mutagenic and/or carcinogenic properties of polycyclic hydrocarbons (for reviews see refs 1-3). Reports^{4,5} suggest that the enzyme plays a dual role in (a) producing proximate carcinogens which, after biotransformation to carcinogens by microsomal mono-oxygenase(s) are (b) inactivated by epoxide hydratase. As this enzyme can be induced⁶⁻⁸, activated⁹⁻¹⁰ and inhibited⁹⁻¹³ it should be useful in studies of the mechanism of chemical carcinogenesis: some inverse correlations have been reported between susceptibility to carcinogenesis by polycyclic hydrocarbons and inducibility of epoxide hydratase at different stages of development in the rat¹⁴. Most investigations of this enzyme^{6-10,15-18}, however, have involved radiometric assay with ³H-styrene oxide as substrate¹⁸. There is evidence that the enzyme(s) responsible for the hydration of this substrate are also involved in hydration of epoxides, derived

from polycyclic hydrocarbons^{8,19}, while other evidence suggests the presence of several epoxide hydratases in the microsomal membrane^{7,9,10,19}. Thus it was not known whether information about epoxide hydratase obtained with styrene oxide as substrate applied to the enzyme(s) responsible for hydration of epoxides derived from polycyclic hydrocarbons. Using antibodies raised against a homogeneous epoxide hydratase preparation and other criteria described here, we have now found evidence that intact microsomal membranes contain a single enzyme able to hydrate styrene oxide and benz(a)pyrene, 4,5-oxide, a K-region epoxide.

Epoxide hydratase was solubilised from adult male Sprague-Dawley rat liver microsomes and purified to homogeneity as established by gel electrophoresis, analytical ultracentrifugal and immunological criteria²⁰. Antibodies to this preparation were raised in New Zealand white rabbits. The animals were immunised intradermally at several sites along the flank with 100 µg of pure epoxide hydratase in Freund's complete adjuvant. Booster injections of 50 µg of protein in 50 mM Na phosphate buffer were given intradermally 8 and 16 weeks later. Rabbits were bled and serum was collected 8–10 d after the last injections. A 200 µl sample of either crude epoxide hydratase solution (solubilised microsomes, 1.4 mg protein) or homogeneous epoxide hydratase (46 µg) was kept for 18 h at 0–4 °C in the presence of a constant amount (200 µl) of serum containing various amounts of antiserum. Controls were kept in the same conditions but in the presence of control serum. Precipitated antigen-antibody complexes were sedimented by centrifugation at 8,000g for 20 min and supernatant fractions were assayed for epoxide hydratase activity in the absence of Tween 80 with substrates styrene oxide¹⁰ and benz(a)pyrene 4,5-oxide (H. U. Schmassmann *et al.*, unpublished). To determine the inhibitory potency of the antibodies, enzyme preparations were kept in the presence of antiserum for 15 min at room temperature and then assayed without previous centrifugation. In the experiments with low molecular weight inhibitors, these were added to the incubation mixture at zero time with no preincubation, in 10 µl of acetonitrile with styrene oxide and in 25 µl of acetonitrile with benz(a)pyrene 4,5-oxide as substrates, while controls had the same amounts of acetonitrile.

Figure 1 shows the results of an immunoprecipitation

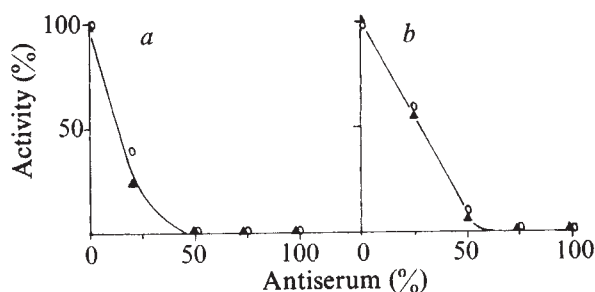


Fig. 1 Immunoprecipitation of epoxide hydratase activity with styrene oxide (▲) and benz(a)pyrene 4,5-oxide (○) as substrate in crude hydratase solution (solubilised microsomes) (a), and homogeneous hydratase (b) by antibodies raised against an epoxide hydratase preparation homogeneous by gel electrophoresis, analytical ultracentrifugal and immunological criteria. The results are expressed as percentage of the activity of samples treated similarly with serum from control animals. These control activities were 10.6 and 432 nmol styrene glycol and 3.7 and 136 nmol benz(a)pyrene 4,5-dihydrodiol per min per mg protein with the crude hydratase solution and the homogeneous hydratase, respectively. Differences between these controls and samples without serum protein were always less than 30% and were noticed only when homogeneous epoxide hydratase was used, probably because the protein content present in those assay mixtures was extremely low in the absence of serum proteins.

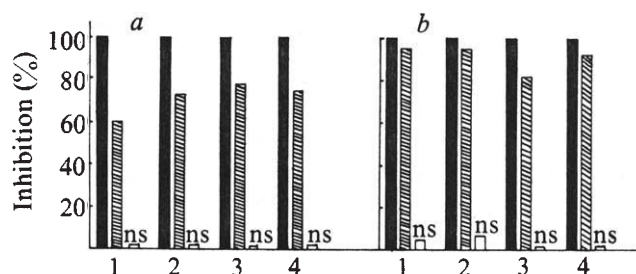


Fig. 2 Inhibition of epoxide hydratase activity with 2 mM styrene oxide (a) and 0.15 mM benz(a)pyrene 4,5-oxide (b) as substrate by 2 mM 1,1,1-trichloropropene 2,3-oxide (■), 2 mM cyclohexene oxide (▨) and 2 mM *trans*-stilbene oxide (□). Enzyme preparations were: 1, rat liver microsomes; 2, solubilised microsomes; 3, phosphocellulose column effluent; 4, homogeneous epoxide hydratase. The specific activities of the various preparations reflecting their relative purification were: 1, 5.01; 2, 8.91; 3, 260; 4, 600 nmol styrene glycol per min per mg protein. Inhibitors were added at zero time in acetonitrile solution. Controls received the same amount of acetonitrile. Results are expressed as percentage inhibition compared with such controls. ns, not significant (level of significance: $P < 0.05$).

experiment. Antibodies raised against homogeneous epoxide hydratase, which had been purified²⁰ using an assay with styrene oxide as substrate¹⁰, precipitated epoxide hydratase activity towards both styrene oxide and benz(a)pyrene 4,5-oxide. Precipitation, which was dose-dependent, occurred when antiserum was incubated with either the pure enzyme or the crudest soluble preparation (solubilised microsomes). Immunoprecipitation was complete at the higher concentrations of antiserum. This suggested the presence of a single enzyme responsible for the hydration of the two substrates. On the other hand, there may be several immunologically cross reactive epoxide hydratases or some epoxide hydratase species may be inactivated differentially but completely during solubilisation of the microsomes. But although the extent of inhibition of epoxide hydratase activity by antiserum was different in microsomes (20–30%) compared with soluble preparations (70–80%), inhibition of the hydration of the two substrates was always similar ($\pm$ less than 10% of the mean) (data not shown). Furthermore, the relative potency of low molecular weight epoxide hydratase inhibitors remained essentially the same throughout purification. This was true for either substrate. As Fig. 2 shows, when styrene oxide was used as substrate the membrane-bound enzyme (1), the crudest solubilised preparation (2), a partially purified preparation (3), and homogeneous epoxide hydratase (4), were all inhibited completely by 1,1,1-trichloropropene 2,3-oxide, very strongly ($\sim$ 60–80%) by cyclohexene oxide and not significantly ($< 10\%$) by *trans*-stilbene oxide. Epoxide hydratase activity with benz(a)pyrene 4,5-oxide as substrate was also inhibited by these agents to very similar extents in the different enzyme preparations. Finally, only a single side fraction during purification had detectable epoxide hydratase activity towards either of the substrates used. The ratio of the specific activities towards the two substrates was the same in this single side fraction as in the fraction containing the bulk of epoxide hydratase activity.

These results, taken together, strongly suggest that a single enzyme is involved in the hydration of both styrene oxide and benz(a)pyrene 4,5-oxide in rat liver microsomes. Thus, information already acquired about epoxide hydratase, using styrene oxide as substrate, seems to be directly relevant to the enzymatic entity responsible for hydration of benz(a)pyrene 4,5-oxide, a K-region epoxide and, most probably, many epoxides derived from several carcinogenic polycyclic hydrocarbons.

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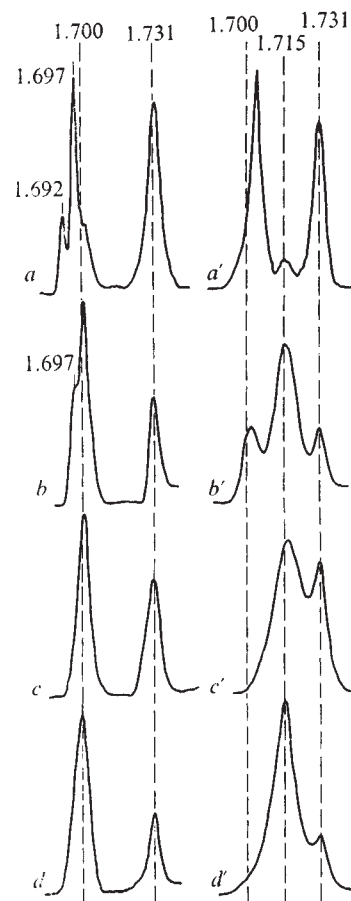


Fig. 1 Buoyant density pattern of *Ascaris* DNA centrifuged in neutral CsCl. Left column, native DNA; right column, denatured unsheared DNA reassociated to C_0t 0.5 mol nucleotide s^{-1} . a, a', Spermatid germline DNA of *Parascaris equorum* race *univalens*; b, b', spermatid germline DNA of *Ascaris lumbricoides* var. *suis*; c, c', larval soma DNA of *P. equorum*; d, d', larval soma DNA of *A. suis*. Marker DNA was from *Micrococcus lysodeikticus* with density taken as 1.731 $g\ cm^{-3}$, implying an *E. coli* DNA density of 1.710 $g\ cm^{-3}$ in our analytical centrifugations. Mitochondrial DNA of *Ascaris* bands at 1.686 $g\ cm^{-3}$ (ref. 11). For further explanation see text.

Complexity of germline and somatic DNA in *Ascaris*

DURING the early determination of the blastomeres in the nematode *Ascaris* chromosomal segments are eliminated in the pre-somatic cells (chromatin diminution). In the germline, however, all the chromatin is preserved. Similar genome differentiation phenomena have been published^{1–3}. Since no molecular description of chromatin diminution has been made we have begun to study the chromosomes in germline and somatic cells⁴. It was found that the amount of DNA occurring only in the germline chromosomes is not constant⁵ and that this DNA is not enriched in rDNA⁶. There is no evidence that the germline-limited DNA is transcribed *in vivo*⁷. The results presented here show that the germline DNA of *Ascaris* contains highly repetitive components, eliminated by the diminution process. Their possible molecular function in connection with germline-soma differentiation and chromosomal behaviour is discussed.

By means of Feulgen photometric measurements and Burton's reaction, we determined the DNA content of germline and soma genomes of *Parascaris equorum* race *univalens* as 1.2–2.1 pg and 0.25 pg, and *Ascaris lumbricoides* var. *suis* as 0.32 pg and 0.25 pg, respectively. Therefore, *Parascaris* contains up to 85% germline-limited DNA but *A. suis* only 22%. An analysis of melting curves had shown that the G+C content of the germline DNA differs from that of soma DNA⁸. Buoyant density profiles of DNA in neutral CsCl gradients demonstrate the presence of germline satellites in both species (Fig. 1). Germline DNA of *P. equorum* contains two satellites of sizes unique in the animal kingdom, with densities 1.697 $g\ cm^{-3}$ and 1.692 $g\ cm^{-3}$ (Fig. 1a); the dense shoulder at 1.700 $g\ cm^{-3}$ represents the single copy DNA. After denaturation and reassociation to low C_0t (Fig. 1a') the highly repetitive components (satellites) form one peak at 1.703 $g\ cm^{-3}$, making up about 85% of the total germ-

line DNA. The single copy DNA, still denatured, forms the side peak at 1.715 $g\ cm^{-3}$. The sharp profile of the reassociated fraction may indicate short basic nucleotide sequences of the satellite DNA. Since only one peak is generated one might deduce that there is a sequence relationship between the two satellites. In *A. suis* the satellite DNA at 1.697 $g\ cm^{-3}$ (Fig. 1b) is not sharply separated from the main band DNA as reported earlier⁹. After denaturation and reassociation to low C_0t , however, the single-stranded DNA has shifted away from the reassociated satellite DNA (Fig. 1b'), which is considered to be about 23% of the germline DNA. In the native larval somatic DNA of both species (Fig. 1c and d) satellites are absent. Apparently they have been lost during chromatin diminution. Even after density transposition of single copy DNA by denaturation and following low C_0t reassociation there is no evidence for satellite DNA (Fig. 1c' and d'). If we incubate denatured unsheared soma DNA of *A. suis* to low C_0t and subsequently digest it by single-strand specific endonuclease, 4% is revealed as enzyme resistant on CsCl centrifugation.

Reassociation kinetics (Fig. 2) of total genome DNA of *A. suis* demonstrate that 17.5% of testis DNA (predominantly germline DNA) consists of repeated sequences whereas the larval soma DNA does not contain any measurable highly repetitive component. This latter finding contradicts the data of Tobler *et al.*¹⁰ who reported a value of 10% repetitive DNA

for soma DNA. In spermatid DNA which is not contaminated by follicle epithelium DNA and contains only insignificant amounts of mitochondrial DNA repetitive sequences make up 20–22% (refs 8 and 10).

The slowly-reassociating DNA of germline and of soma reassociates homogeneously with second order kinetics (Fig. 2), and the reassociation rate constants correspond well. This indicates that there is identical single copy DNA complexity in the germline and the soma. The $C_0t_{1/2}$ values corrected for 50% G+C (ref. 11) suggest that the somatic DNA as well as the plurivalent germline chromosomes constitute the unit genome¹² since their analytical values compare well with the reassoci-

Table 1 Comparison of genome sizes and kinetic complexity in daltons

DNA (% G+C)*	Analytical value	Kinetic value†
<i>E. coli</i> (50)	2.7×10^9	
<i>A. suis</i> soma (40.8)	1.5×10^{11}	1.9×10^{11}
<i>A. suis</i> germline (40.8)	1.9×10^{11}	2.2×10^{11}
<i>Gallus domesticus</i> (42)	$7.5 \times 10^{11} \ddagger$	5.9×10^{11}

*G+C content of single copy DNA.

†Corrected for G+C content and proportion of fast reassociating components.

‡Ref. 13.

ation kinetic values using *Escherichia coli* DNA as a standard, within the limitations of the method (Table 1). Consequently, in the germline genome there is no room for any appreciable amount of germline-limited single copy DNA (estimated to be one half of germline-limited DNA by ref. 10), which could be detectable by means of reassociation kinetics studies. This is independently confirmed by the agreement of the two percentages; the germline-limited DNA (22%) and the fast-reassociating DNA fraction (20–22%) of the spermatid (that is, germline) genome. If one considers both the cytology of diminution and that the soma DNA does not contain satellite DNA then the identity of those two percentages shows that the reiterated sequences are clustered.

We have repeatedly measured the reassociation of the more complex single copy DNA of *Gallus domesticus*. Using *E. coli* DNA as a standard, the $C_0t_{1/2}$ value was always estimated to be

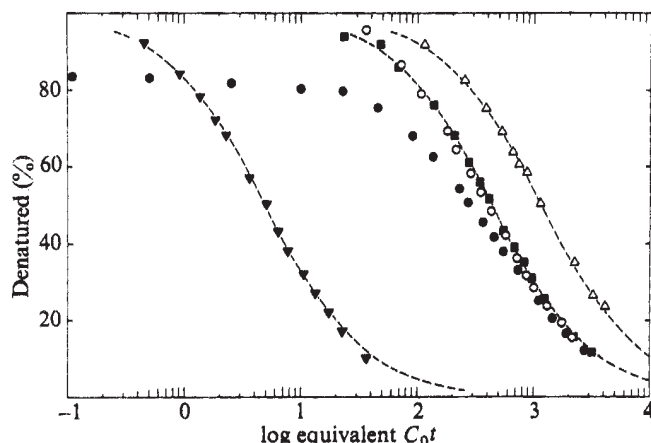


Fig. 2 Reassociation kinetics, analysed optically, of larval soma DNA (■), of total testis (germline) DNA (●) and single copy testis DNA (○) of *A. suis*, of *E. coli* DNA (▼) as a standard and of single copy DNA of *Gallus domesticus* (△). Larval DNA was extracted from embryos, testis DNA from whole gonads. The DNA isolation involved proteinase K (Merck) digestion of the lysates, deproteinisation by phenol, pancreatic RNase and T₁ RNase digestion; and hydroxyapatite column chromatography. DNA was sonicated in 0.01 M Tris-HCl pH 7.1 to a mean fragment size of 400 nucleotide pairs. Reassociation buffer was 0.01 M Tris-HCl, 2 M NaClO₄ pH 7.1. After melting of the DNA in a water-jacketed cuvette (pathlength 1 mm) the temperature was lowered within seconds to incubation temperature (25 °C beneath T_m). The absorbance was recorded by means of a double beam equipment. Timed absorbance values were stored with an interface in a computer. For the description of the second order reaction the following equation was taken

$$(\Delta_t^{\text{ren}})^{-1} = (\Delta_0^{\text{ren}})^{-1} + kt$$

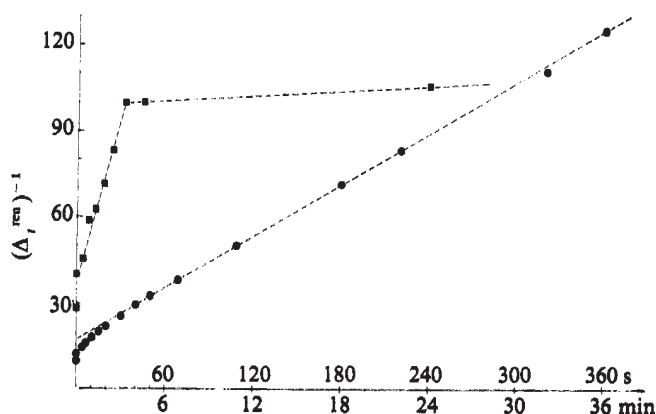
$$\begin{aligned} \text{with } \Delta_t^{\text{ren}} &= A_t - A_{\infty}^{\text{ren}} \\ \Delta_0^{\text{ren}} &= A_0^{\text{ren}} - A_{\infty}^{\text{ren}} \\ k &= (\Delta_0^{\text{ren}} \times t_{1/2})^{-1} \end{aligned}$$

A_t is the absorbance at time t , A_0^{ren} that after deducting collapse hypochromicity¹³, A_{∞}^{ren} that for infinite reaction, which is approximated by linear regression for the time interval within which 75% of the single copy DNA has reassociated. From the regression line the C_0t curves were obtained using:

$$\Delta_t^{\text{ren}} \times (\Delta_0^{\text{ren}})^{-1}$$

as fraction of denatured DNA. C_0t was transformed to Ec_0t (ref. 16) (incubation buffer 0.12 M phosphate). In 2 M NaClO₄ reassociation is 5.5 times faster than in 0.12 M phosphate. The dashed lines are theoretical second order curves. For *E. coli* DNA $Ec_0t_{1/2} = 4.8$. The germline DNA from testis contains 17.5% fast reassociating sequences. The C_0t curve of germline single copy DNA ($Ec_0t_{1/2} = 410$) was evaluated from the curve of total testis DNA. For larval soma DNA $Ec_0t_{1/2} = 440$. For single copy chicken DNA $Ec_0t_{1/2} = 1,190$. DNA concentrations were 461 $\mu\text{g ml}^{-1}$ for *E. coli*, 772 $\mu\text{g ml}^{-1}$ for *Ascaris* testis, 1,172 $\mu\text{g ml}^{-1}$ for *Ascaris* larva, and 862 $\mu\text{g ml}^{-1}$ for *Gallus*.

Fig. 3 Reassociation, measured by hypochromicity, of isolated highly repetitive components of soma and germline DNA of *A. suis*. Sheared larval soma DNA and testis DNA were denatured and incubated to $Ec_0t = 3$. The reassociated DNA was bound on to a 60 °C hydroxyapatite column equilibrated in 0.12 M phosphate, the absorbed fraction eluted by 0.35 M phosphate washing. Soma DNA yields a bound fraction between 3.5–6.5%, testis DNA about 14%. Reassociation of fast renaturing sequences was done in 0.03 M phosphate, where reassociation is 75 times slower than in 0.12 M phosphate¹⁶. Reassociation was recorded in double beam using water-jacketed cuvettes (10 mm pathlength) and temperature measuring equipment in the reference cuvette. In the experiment shown, the soma DNA fraction (■), contaminated by single copy DNA, renatured to 55%, of which 35% (2.3% of total soma DNA) occurred within 30 s. For this fraction Δ_t^{ren} was 0.16, hence at $Ec_0t = 10^{-3}$ this fraction (foldback DNA) had completely renatured. The other reassociating fraction makes up 20% (1.3% of total soma DNA). The reassociation of the highly repetitive testis DNA (●) may be interpreted as the reaction of three components: one reassociating with $t_{1/2} = 5.7$ min and $\Delta_0^{\text{ren}} = 0.057$, thus yielding an $Ec_0t_{1/2}$ of 2×10^{-4} , making up some 70% of the fast renaturing sequences. The additional 30% ($\Delta_0^{\text{ren}} = 0.024$) contain 13% (2.3% somatic foldback DNA correspond to 1.8% in the germline; 1.8%:14%) foldback DNA and 17% highly repetitive sequences. For symbols see legend of Fig. 2. Abscissa: time of incubation; in s for soma DNA, in min for germline DNA.



smaller than expected from the analytical genome size for which divergent values have been published, however.

In order to characterise the repeated DNA sequences, they were isolated from total DNA. Reassociation to low C_0t of denatured sheared total larval soma DNA of *A. suis* (Fig. 3) yields a renatured fraction of approximately 4%. This was isolated as 0.35 M phosphate buffer fraction by HAP column chromatography using 0.12 M phosphate as the discriminating concentration between single and double-stranded fragments. The proportion of the fast-renaturing DNA is 2.3% of the total DNA. It has completely renatured at $Ec_0t < 10^{-5}$, and has a sharp melting profile. Its hyperchromicity, however, is low. Its broad banding profile in neutral CsCl reveals its network configuration; it is also highly resistant to endonuclease. These are all characteristics of foldback DNA¹⁴. Continued reassociation, after the foldback DNA has completely renatured, reveals a slowly reassociating component which is about 1% of the total DNA. Its percentage is small compared with that found in other organisms. If one accepts that intermediate repetitive sequences have a regulatory function¹⁵, the small amount present in *Ascaris* reflects the simple organisation of this organism.

From denatured sheared testis (that is, germline) DNA after reassociation to low C_0t the reassociated fraction isolated by HAP chromatography makes up 14% of the total DNA. Its reassociation indicates fast reassociating sequences in addition to the ubiquitous foldback DNA. Their reassociation curve (Fig. 3) may be interpreted as the reaction of three components: the foldback DNA and two highly repetitive classes of DNA: One makes up 21% of the germline-limited DNA which reassociates with $C_0t_{1/2} < 10^{-4}$, the other predominant one (79%) reassociates with $C_0t_{1/2} = 2 \times 10^{-4}$. Alternatively, the initial delay of the reaction, indicated by the continuous decline of the slope of the curve in Fig. 3, may be caused by increasing hyperpolymerisation¹⁶ during the reassociation of only one component degenerated in its basic nucleotide sequence. In neutral CsCl, the isolated germline-limited DNA forms one sharp peak. This, together with its high hyperchromicity, indicates its highly native state after reassociation. Its T_m is 3 °C lower than that of the foldback DNA.

The observations on the abnormal cleavage of the *Ascaris* egg¹⁷ have shown that the existence of germline limited chromatin is a sufficient criterion of the germline quality of a blastomere; the irradiation of germline blastomeres¹⁸ showed that the existence of that chromatin is necessary. The data presented here demonstrate, for the first time for an organism with germline-limited chromatin, that the presence of highly repetitive DNA components in itself is sufficient for the genetic status of a germline cell. Yet, in spite of the developmental significance of this type of chromatin, the molecular function of its highly repetitive DNA components remains obscure.

But three properties of the *Ascaris* germline chromosomes may be related to this function. (1) The highly repetitive DNA sequences are components of the heterochromatic sections of the germline chromosomes. After diminution the chromosomes are euchromatic. Therefore, these sequences might possibly suppress the activity of somatic genes, as happens in position effect variegation, and thus be responsible for the maintenance of the germline quality. (2) Germline chromosomes have a tendency to form plurivalent chromosomes. In extreme cases, the entire genome is integrated into a single plurivalent chromosome. The tandem reiteration of distally localised sequences allows the formation of sticky ends and, consequently, the linking together of chromosomes. Aggregation of the minute somatic single chromosomes to large plurivalent chromosomes prevents mistakes in meiotic distribution. (3) The germline chromosomes are holocentric⁴. The germline-limited chromatin has kinetic activity in anaphase. This property, which is lost in preparation for diminution, increases the chromosome manoeuvrability. This latter must be guaranteed, especially in parasitic organisms such as *Ascaris* which produce exceedingly large quantities of gametes. These observations in *Ascaris* and

their interpretations support quite well Walker's ideas¹⁹ on the significance of highly repeated sequences.

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No detectable satellite DNA in supernumerary chromosomes of the grasshopper *Myrmeleotettix*

SUPERNUMERARY (B) chromosomes and satellite (simple sequence) DNA are enigmatic chromosomal phenomena, in that both are variable additions to the main chromosome complement. It has been suggested that differences between, and constancy within, certain related species for satellite DNA, implies that it may play a role in speciation by a series of saltatory steps^{1–3}. Similarly, B chromosomes may affect recombination⁴ or rate of development⁵, or bring about diploidising effects in hybrids of closely related species⁶. There is still, however, no clear understanding of the functions of either of these variables.

The first report of between and within-population variation in satellite DNA, associated with the presence or absence of B chromosomes in the grasshopper *Myrmeleotettix maculatus*^{7,8}, suggested that there might be a closer relationship between satellite DNA and supernumerary chromosomes. Analysis of sedimentation patterns of total DNA in neutral caesium chloride showed that the DNA of B chromosome-containing individuals, from several different populations, contained satellite DNA not found in individuals lacking B chromosomes. Furthermore, both main-band and satellite DNA were found to vary in buoyant density in populations from different localities.

Several studies, however, on the heterogeneity of B chromosome DNA in plants^{9–11}, have failed to reveal large fractions of highly repetitive or moderately repetitive DNA exclusive to the B chromosomes. Detailed examination of several parameters of base composition of A and B chromosome DNAs were made in the wheat group of species¹¹. These included profiles of thermal dissociation of homologous and heterologous duplexes, differential melting curves in strictly comparable den-

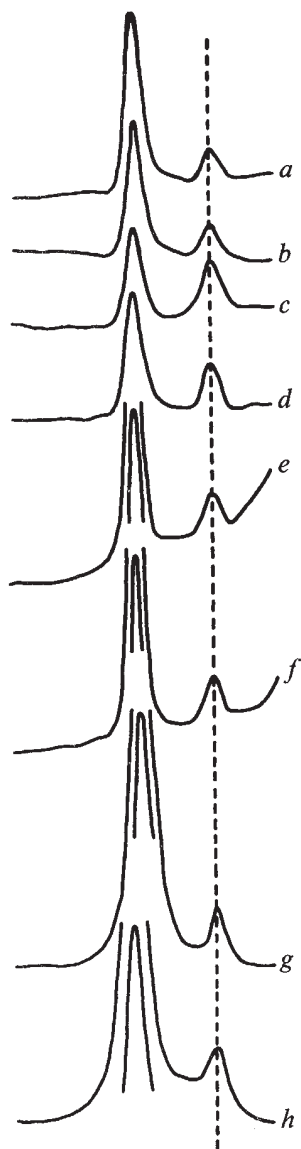


Fig. 1 Analytical ultraviolet absorbance patterns after sedimentation to equilibrium in neutral CsCl for 20 h at 44,000 r.p.m. at 25 °C on a Centriscan 75 analytical centrifuge. *a-f*, Pronase-digested lysates of testes or ovaries of single individuals. Tissue was lightly ground in glass homogenisers in 150 μ l of 0.5% sodium lauryl sulphate, 0.1 M EDTA, 0.05 M Tris, pH 8.4 and lysed for 2 h at room temperature. After lysis, 0.5 ml of 10 mM Tris-HCl, pH 7.8 and 100 μ l of self-digested Pronase (1 mg ml⁻¹) were added and incubated for 2 h at 37 °C. Solid CsCl (ultra-centrifuge grade) was added to an initial density of 1.700 g cm⁻³. A sample of 2 μ l of marker DNA ($A = 4$) of *Pseudomonas aeruginosa* (density = 1.726 g cm⁻³) was added. *g-h*, Bulk tests of 25 individuals were homogenised in 4 M CsCl (initial density = 1.4 g cm⁻³) in a glass homogeniser. The homogenate was centrifuged in a 8 $\times$ 14 ml rotor of an MSE 65 centrifuge for 20 h at 40,000 r.p.m. at 25 °C. The pellet was dissolved in 4 ml of 1 $\times$ SSC (0.15 M NaCl + 0.015 M Na citrate) and incubated at 37 °C successively with α -amylase (250 μ g ml⁻¹) for 1–2 h, RNase (250 μ g ml⁻¹, preheated to 80 °C for 5 min) for 1–2 h, and Pronase (1 mg ml⁻¹, self-digested at 37 °C for 2 h) for 1–2 h. Insoluble material was removed with low speed centrifugation and the DNA was pelleted for 1–2 h at 40,000 r.p.m. at 4 °C and dissolved in 10 mM Tris-HCl, pH 7.8. Conditions for analytical ultracentrifugation were as in *a-f*. *a*, Southwold δ (no B); *b*, Mildenhall δ (no B); *c*, Mildenhall δ (one B); *d*, Mildenhall δ (two B); *e*, Foxhole δ (no B); *f*, Foxhole δ (one B); *g*, Mildenhall δ (bulk extract) (no B); *h*, Mildenhall δ (bulk extract) (one B).

aturation conditions and equilibrium sedimentation patterns in neutral CsCl and actinomycin D–CsCl gradients. Within the limits of resolution of these experiments, it has been shown that the B chromosomes contain representatives of the spectrum of repeated sequence families present on the A chromosomes: there are no discrete B chromosome fractions of DNA which form satellites, or which are not homologous to A chromosome DNA when they are hybridised together.

Although these studies demonstrate that the presence of distinct satellite DNA is not a general property of B chromosomes *per se*, the *Myrmeleotettix* satellite could well reflect the origin and composition of that particular B chromosome system. The large, varying amounts of satellite demonstrated in this grasshopper, however, were far greater than expected from the measured size of the B chromosome. The unusually large variations in main-band and satellite buoyant densities from different populations are also difficult to explain. As part of a general study of interpopulation differences of satellite DNA in Orthoptera and *Drosophila*, the anomalous situation in this grasshopper has been re-examined.

Male and female adult individuals of *Myrmeleotettix maculatus* were collected during August and September 1975 from wild East Anglian populations in the Mildenhall/Foxhole Warren area, where a 50% B chromosome frequency is found. Others were collected from a coastal population near Southwold, where no B chromosomes occur. Testes and ovaries were vivisected in cold isotonic saline and cleaned of fat body. Part of each testis or ovary was placed in acetic alcohol fixative before determination of the B chromosome status in propionic orcein squash preparations. The remainder was used fresh for DNA extraction or stored in a deep freeze until required. Females were injected with colcemid 1 h before dissection, to facilitate the analysis of ovariole mitoses.

DNA extracts were obtained both from single individuals and from the bulk extraction of the homogenised gonads of 25 individuals with, and 25 without, B chromosomes. Details of the two methods are given in the legend to Fig. 1. The methods of extraction were identical to those already published^{7,8}, to ensure direct comparability of results. Pronase-treated lysates of individual gonads were loaded directly into analytical cells of an MSE Centriscan centrifuge, in neutral CsCl. The DNA of bulked gonads, after removal of protein, polysaccharide and RNA contaminants, was sedimented to equilibrium in neutral CsCl and actinomycin D–CsCl gradients. All gradients were scanned with Schlieren visible light optics to detect the presence of glycogen and other ultraviolet (254 nm) and visible light (540 nm) absorbing contaminants. If present, such extraneous ultraviolet-absorbing peaks have been removed from the traces for clarity.

A selected range of ultraviolet absorbance profiles of grasshopper DNA, after sedimentation to equilibrium in neutral CsCl, are shown in Fig. 1. No satellites to the main band were found in any of the grasshoppers with 0, 1 or 2 B chromosomes, of either sex, in any population sampled. The main-band buoyant densities and the overall mean buoyant density and standard error are given in Table 1. No significant differences were observed between genotypes or between populations.

Profiles of grasshopper DNA after sedimentation to equilibrium in actinomycin D–CsCl gradients showed a skewed spread of main-band DNA, indicating considerable heterogeneity in base composition (Fig. 2), causing differential binding of the antibiotic to fractions of varying GC content. No main-band cryptic satellites nor changes in main-band skew or spread were revealed in such gradients in the presence of B-chromosome DNA. It is interesting that direct loading and analytical centrifugation of comparable lysates of individual testes,

Table 1 Buoyant densities of main-band DNA

Population	Sex	B-chromosome constitution	Main-band buoyant density (g cm ⁻³)
<i>Single individuals, direct lysates</i>			
Southwold	♂	0	1.7004
Southwold	♂	0	1.7000
Mildenhall	♂	0	1.7010
Mildenhall	♂	1	1.7009
Mildenhall	♂	1	1.7001
Mildenhall	♂	2	1.7004
Mildenhall	♂	2	1.7002
Mildenhall	♂	2	1.7003
Foxhole Warren	♀	0	1.6998
Foxhole Warren	♀	0	1.7007
Foxhole Warren	♀	0	1.7002
Foxhole Warren	♀	1	1.7003
Foxhole Warren	♀	1	1.7004
Foxhole Warren	♀	1	1.7004
Foxhole Warren	♀	1	1.7001
Foxhole Warren	♂	1	1.7002
<i>Bulk extract from 25 individuals</i>			
Mildenhall	♂	0	1.6986
Mildenhall	♂	1	1.6986
Mean =			1.7001 ± 0.00014

Buoyant densities of main-band DNA of testes or ovaries of single individuals or bulked individuals with or without B chromosomes, were calculated¹² relative to the marker *Pseudomonas aeruginosa* (density = 1.726 g cm⁻³). Mean buoyant density and standard error are given.

ovaries and embryos of *Locusta migratoria* and *Schistocerca gregaria* in both neutral CsCl and actinomycin D–CsCl gradients have revealed the presence of previously undetected⁸ GC-rich satellites. These satellites have been isolated and further characterised (in preparation).

The remarkable consistency of main-band densities between different individuals and different populations (Table 1), allows for some confidence to be placed in the procedures used. The lack of a resolvable satellite (Fig. 1) in the presence of B chromosomes in both males and

females throws some doubt on the reported B chromosome–satellite DNA correlation in this species. The present results suggest considerable similarity in base composition of A and B chromosome DNA, as found in several plant species^{9–11}.

It is difficult to explain the presence of satellite DNA and the variation in satellite and main-band base composition previously reported. An exclusion of B-chromosome responsibility for the presence of the satellite leaves the possibilities of either an unrelated form of intra-population variation for satellite, or a pathogen or glyco-gen contamination.

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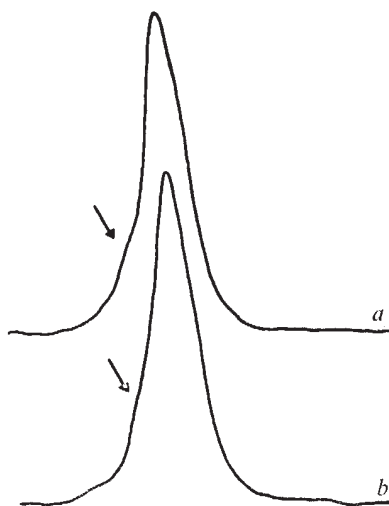
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Fig. 2 Analytical ultraviolet absorbance patterns in actinomycin D–CsCl gradients of DNA of bulked testes with or without B chromosomes, after sedimentation to equilibrium for 20 h at 44,000 r.p.m. at 25 °C. DNA and actinomycin D were dissolved in a ratio of 1:2 in 0.5 ml of 10 mM Tris-HCl, 1 mM EDTA, pH 6.8 and brought to an initial density of 1.660 g cm⁻³ with solid CsCl (ultracentrifuge grade). *a*, From bulked testes of 25 individuals without B chromosomes; *b*, from bulked testes of 25 individuals with one B chromosome. Note absence of cryptic main-band satellites and increased spread and skew of main-band DNA.



Mechanism of differential Giemsa method for sister chromatids

THE 33258 Hoechst fluorescence¹ and 33258 Hoechst–Giemsa² methods for differential staining of bromodeoxyuridine (BrdU)-incorporated chromatids are attractive because of their rapidity and high sensitivity in revealing small sister chromatid exchanges which escape detection by autoradiography. Since carcinogen-induced chromatid breaks are associated with sister chromatid exchanges³ and breakage formation parallels carcinogenesis in typical cases^{4,5}, these methods are essential for understanding the role of chromosome aberrations in carcinogenesis. We show here that other photosensitive dyes such as thionin can replace 33258 Hoechst in the latter method and that the mechanisms of the differential Giemsa method involves photolysis of the BrdU-substituted DNA combined with these photosensitive dyes.

Fresh bone marrow cells from Long–Evans rats were cultured for two rounds of cell cycle or 26 h in thymidine-free F12 medium containing 10% foetal calf serum and 2 µg ml⁻¹ BrdU. Colchicine was added 2 h before collection. Cells were collected, treated with 0.075 M KCl, fixed in 1:3 acetic methanol, spread and air dried on glass slides. Our basic procedure consisted of 33258 Hoechst treatment, exposure to light and Giemsa staining. In addition to 33258 Hoechst, various metachromatic dyes such as thionin, toluidine blue, methylene blue, and neutral red were used successfully. They were dissolved in distilled water at a concentration of 10⁻³–10⁻⁸ M. Chromosome specimens were dipped in distilled water, treated with the above dye solutions for 10 min, rinsed in water, mounted with 0.16 M sodium phosphate and 0.04 M sodium citrate (pH 7.0)¹, and exposed to strong sunlight (noon, clear summer days) for 5 min–3 h. Thereafter, the coverslips were removed and

the cells were stained for 15 min in 2% Giemsa (Merck) solution in 0.05 M phosphate buffer (pH 6.8). Table 1 indicates that differential chromatid staining depends on the amount of 33258 Hoechst bound and the exposure to light. For routine staining, 10^{-4} M 33258 Hoechst (5 mg per 100 ml) with exposure to sunlight for 15–30 min or 10^{-3} M

Table 1 Influence of 33258 Hoechst and period of exposure to sunlight on differential Giemsa staining of sister chromatids

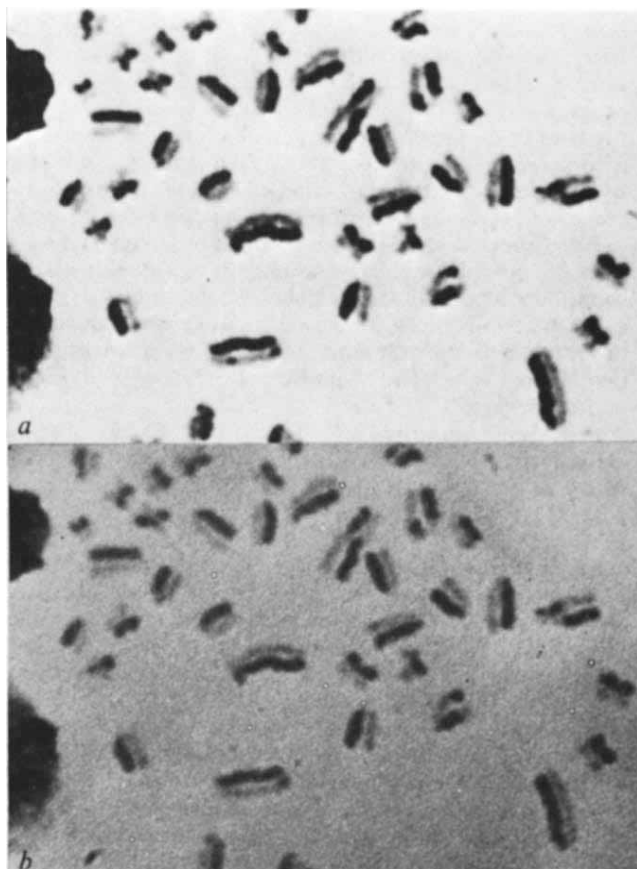
Concentration of 33258 Hoechst (M)	Period (min) of exposure to sunlight						
	5	10	15	30	60	120	180
10^{-3}	++	++	++	++	++	++	++
10^{-4}	++	++	++	++	++	++	++
10^{-5}	±	+	+	++	++	++	++
10^{-6}	—	—	—	+	+	++	++
10^{-7}	—	—	—	—	+	++	++
10^{-8}	—	—	—	—	—	±	+

++, Excellent differentiation; +, moderate; ±, trace; —, negative

thionin (23 mg per 100 ml) with exposure time of 2 h gave an excellent differential staining (Fig. 1a). A longer exposure time was necessary with other dyes. Heating in $2\times$ SSC or in water at 60°C for 2 h (ref. 2) was not essential because differential staining was obtained in the specimens maintained at $20\pm 2^{\circ}\text{C}$ during the whole course of the staining procedure.

Latt suggested that the fluorescence of 33258 Hoechst

Fig. 1 Metaphase bone marrow cell grown for 26 h in BrdU-containing medium. ($\times 3,300$). a, Sister chromatids differentially stained by Giemsa after treatment with 10^{-3} M thionin and 2 h exposure to sunlight. b, Same cell showing differential Feulgen reaction.



bound to the BrdU-substituted chromatids was quenched because the fluorescence efficiency of this dye bound to poly(dA–BrdU) was 20 per cent of that bound to poly(dA–dT)¹. Ikushima and Wolff² and Perry and Wolff³ considered, however, that this hypothesis is unable to explain their 33258 Hoechst–Giemsa method and supposed that the differential Giemsa staining is caused by binding of proteins. To test this possibility, chromosome specimens differentially stained with the above 33258 Hoechst–light–Giemsa method, after destaining in 0.01 M HCl, were treated with 0.01% trypsin in saline at the room temperature for 10–20 s (ref. 7) and restained with 2% Giemsa. This caused G-banding of the darkly Giemsa-stained chromatids but failed to re-establish the affinity of the dully stained chromatids to Giemsa dye. Therefore, proteins do not seem to be involved in the differential staining. On the other hand, Feulgen reaction⁴ carried out on the specimens treated with 33258 Hoechst or thionin and exposed to light gave differential staining (Fig. 1b). This strongly suggests that BrdU-substituted DNA undergoes rapid photolysis in the presence of photosensitive dyes and strong light.

The exact mechanism of the photolysis is unknown. BrdU-substituted DNA, however, is susceptible to light and the BrdU-substituted DNA exposed to light and treated with alkali leads to breaks⁵. On the other hand, the fluorescence of 33258 Hoechst and acridine orange¹⁰ is quenched in the presence of halogenated base analogues possibly by strong magnetic field of the halogen atoms¹¹. Therefore, it is possible that the light-activated dyes situated near the base analogues may act as an alkali causing chain breaks and disorganisation of the chromatid DNA. Further studies with purified BrdU-substituted DNA is in progress.

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Thermolabile transfecting DNA from temperature-sensitive mutant of phage $\phi 29$

THE chromosome of bacteriophage $\phi 29$, one of the smallest *Bacillus* phages, is a non-permuted linear duplex DNA molecule of molecular weight 11×10^6 (refs 1 and 2) that seems to be circularised by association with a protein, as circular monomers are converted into linear monomers by treatment with proteolytic enzymes³. A similar circular DNA–protein complex has been reported for adenoviruses⁴.

DNA extracted from phage $\phi 29$ is infectious (transfectious) when added to competent cells of *B. subtilis*⁵. This transfectivity is sensitive to proteolytic enzymes⁶, indicating that some protein is associated with $\phi 29$ DNA and is required for transfection. Protease-sensitive transfection has also been observed with DNAs of *B. subtilis* phages, GA1 (ref. 7), SPO2 and $\phi 105$ (H. Hirokawa, personal communication).

The biological function of the $\phi 29$ protein required for transfection is not known. It has been shown¹ that genetic markers on half- and quarter-length $\phi 29$ DNA fragments can be rescued using competent cells super-infected with mutant phage, suggesting that DNA-bound protein is not essential for DNA uptake. It has also been shown that protease-treated GA1 phage DNA competes with transforming DNA to the same extent as untreated GA1 DNA⁷. Apparently the protein associated with phage DNA is needed at some stage of transfection other than uptake. Since a specific DNA-bound protein is required for transfection, one might expect to find transfection-defective mutants with altered DNA-protein complexes. Such mutants could aid in defining the role of the protein in DNA transfection. We show here that a temperature-sensitive DNA synthesis mutant of $\phi 29$ originally isolated by Talavera *et al.*^{8,10} produces thermolabile transfecting DNA.

Initially, we anticipated that mutant phages containing a thermolabile DNA-protein complex might be temperature-sensitive for viability. We therefore searched for a $\phi 29$ mutant the viability of which was temperature-sensitive. One of the temperature-sensitive DNA synthesis mutants, *tsK132* (provided by Dr E. Viñuela) was found to be significantly more heat-sensitive than wild type (Fig. 1). Subsequently, we demonstrated that in transfection experiments *tsK132* DNA is also highly thermolabile. Kinetics of the loss of transfectivity as a function of time of heating at 65 °C are shown in Fig. 2. Since transfectivity of DNA samples was determined at 30 °C, the thermosensitivity of *tsK132* DNA is irreversible. Loss of transfectivity at a temperature lower than the melting temperature of $\phi 29$

Fig. 1 Comparison of the heat stability of wild-type $\phi 29$ (○) and *tsK132* phage particle (●). Phages (1×10^6 PFU ml⁻¹) in phage adsorption medium (0.1 M NaCl, 10^{-3} M MgSO₄ in 0.1 M Tris-HCl buffer, pH 7.0) were heated at 70 °C for the indicated times. After dilution, phages were titrated at 30 °C using *B. amyloliquefaciens* H as indicator.

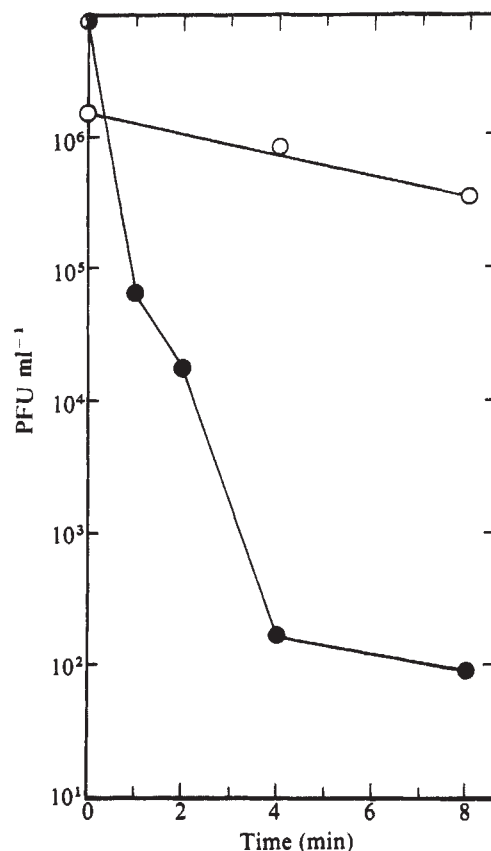
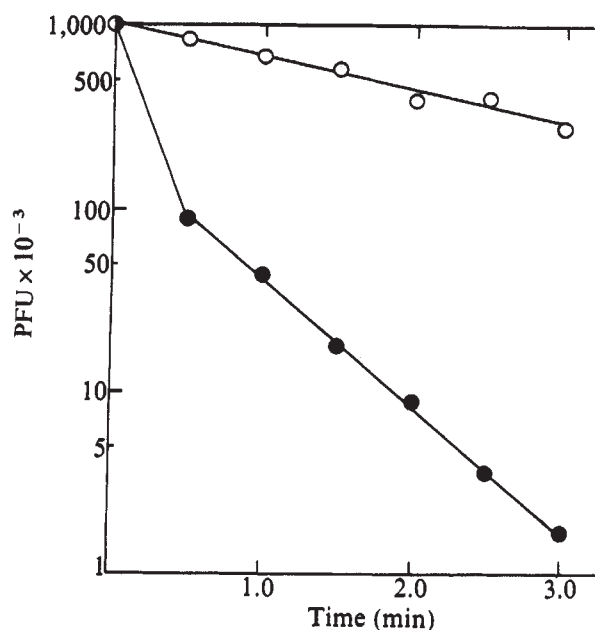


Fig. 2 Comparison of thermostability of transfectious DNA from wild-type $\phi 29$ (○) and *tsK132* (●). Purified phage particles were disrupted by treatment with 1% Sarkosyl for 3 min at 40 °C. Transfectious DNA was phenol extracted and dialysed extensively against standard saline citrate in 0.01 M Tris-HCl buffer, pH 7.0, containing 0.1 mM EDTA. Each DNA solution (0.5 μ g ml⁻¹) was heated for the indicated times and used for transfection. Transfectivity was determined at 30 °C using competent cells of *B. subtilis* 168 according to the method of Reilly and Spizizen⁵.

DNA (T_m 86 °C)¹¹ suggests that this thermosensitivity results from an alteration in the protein associated with the DNA. The *K* gene of $\phi 29$ is one of four genes acting on DNA synthesis early in infection¹². Therefore, it was of considerable interest to establish that the same mutation affects both DNA replication and transfection.

DNA synthesis experiments were carried out to ascertain whether a purified reisolate of *tsK132* was temperature-sensitive for DNA replication. Thymine-requiring cells infected with phages were incubated in a medium containing tritiated thymidine and 6-(*p*-hydroxyphenylazo)-uracil (HPUra), added to inhibit host DNA synthesis^{9,13}. Aliquots were removed at different times for determination of radioactivity in acid-insoluble material. Phage DNA synthesis in *tsK132*-infected cells is almost completely inhibited at 45 °C, whereas it is normal at 30 °C. A shift-up experiment was carried out to determine whether the temperature-sensitive function determined by the *K* gene is required continuously for DNA synthesis or whether it is completed at a definite time during the latent period. Cultures infected and incubated at 30 °C were shifted to 45 °C at different times and DNA synthesis was measured (Fig. 3a). Phage DNA synthesis in cells infected with the mutant stopped immediately when the cells were shifted from 30 to 45 °C. Therefore, the functional product of the *K* gene is required continuously for $\phi 29$ DNA synthesis, and in mutant *tsK132* this product is inactivated immediately at 45 °C.

The results of shift-down experiments (Fig. 3b) indicate

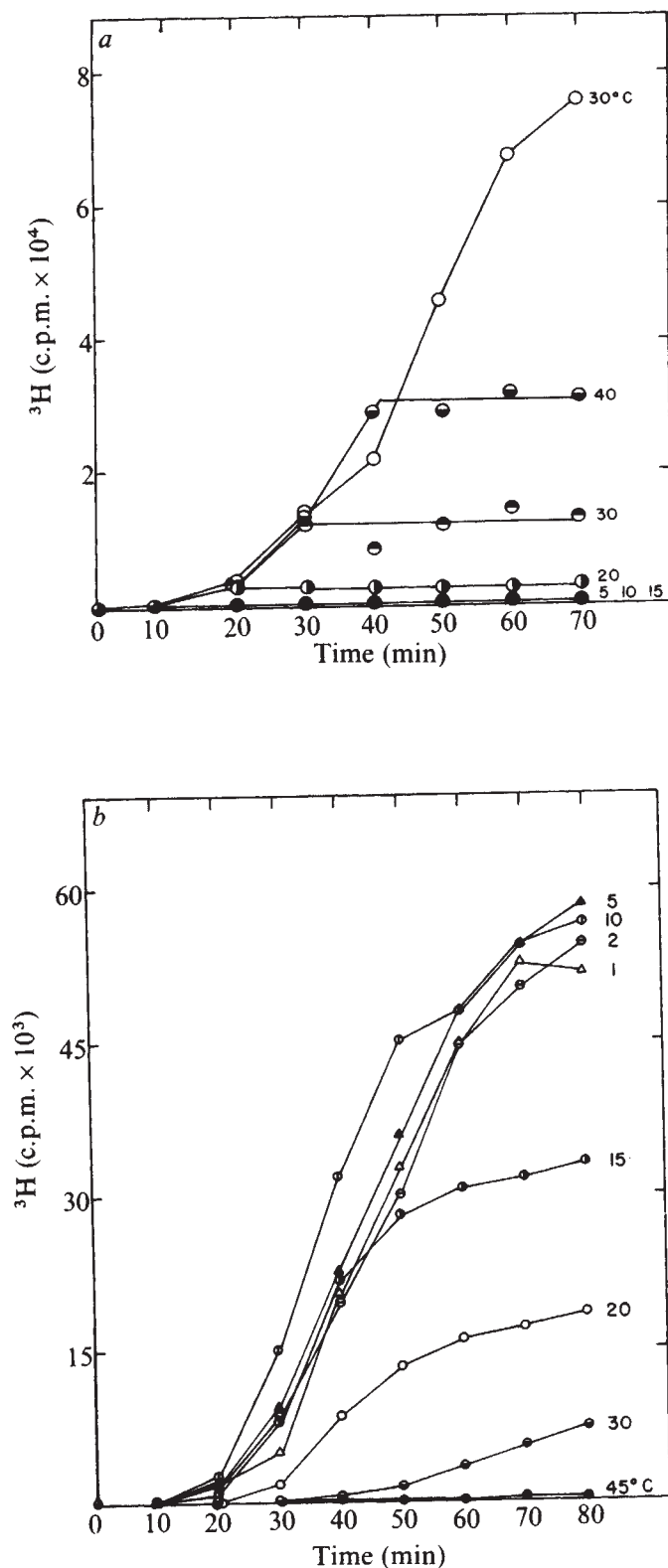


Fig. 3 Effects of temperature shift-up and shift-down on DNA synthesis in *tsK132*-infected cells. *B. subtilis* SCR 115 (*spoA12 thyA*, *B*) was grown to 2×10^8 cells ml^{-1} in Penassay broth containing $2 \mu\text{g ml}^{-1}$ thymine at 37°C , centrifuged, and resuspended in adsorption medium. Cells were mixed with phages at a multiplicity of 20 and incubated for 10 min at room temperature to enable adsorption. Bacteria-phage complexes were then transferred into ^3H -thymidine incorporation medium consisting of Penassay broth, $50 \mu\text{g/ml}$ HPUra and $10 \mu\text{Ci/ml}$ ^3H -thymidine. Cultures were incubated with shaking at either 30°C (a) or 45°C (b). Infected cultures were shifted to the other temperature at the indicated times (min). Samples were removed, treated with 10% cold TCA, filtered, washed with 5% TCA-containing $100 \mu\text{g ml}^{-1}$ thymidine and counted.

that shifts carried out up to 10 min after infection do not affect the ability of cells infected with *tsK132* to synthesise phage DNA. After this time, shift-down results in depressed levels of DNA synthesis. These results agree with those of Talavera *et al.*^{9,10} in showing that mutant *tsK132* is deficient in DNA replication *in vivo*. Since both RNA and protein synthesis are required for $\phi 29$ replication¹⁴, we examined the possibility that phage RNA synthesis is also shut off immediately after shifting the temperature from 30 to 45°C . RNA was extracted from *tsK132*-infected cells at various times after shifting from 30 to 45°C and hybridised to denatured $\phi 29$ DNA immobilised on nitrocellulose filters. The results (not shown) indicate that phage messenger RNA (mRNA) synthesis is not blocked immediately after shifting from 30 to 45°C . Chloramphenicol added before the onset of DNA synthesis inhibits $\phi 29$ DNA replication. It does not, however, shut off DNA synthesis immediately when added after 10 min of infection (not shown). These findings suggest that the immediate cessation of DNA synthesis in *tsK132*-infected cells at non-permissive temperatures is not secondary to an inability to synthesise phage-specific RNA or protein. Rather, it seems more likely that an essential component of the DNA-synthesising machinery is itself thermosensitive.

Reversion studies indicate that the *tsK132* mutant is a point mutation. In four isolated plaques of *tsK132* grown at 30°C , revertants appeared at an average frequency of 5×10^{-6} . Experiments with three *tsK132* revertants showed that they were indistinguishable from the wild type at 30 and 45°C in growth and thermostability of transfecting DNA. One of the revertants was crossed with the wild-type phage and 1,450 progeny phages were tested for temperature sensitivity. No *tsK132* segregants were found. We conclude that *tsK132* is a single-base change mutant and that reversion can occur at or near the site of the original lesion. Our results, therefore, together with those of Talavera *et al.*^{9,10}, show that a single mutation affects both DNA replication and transfection. Consequently, it seems that at least one DNA-associated protein contained in the phage particle is involved in $\phi 29$ DNA transfection. We have not yet identified the protein.

The possibility that the *K* protein is involved in circularisation of $\phi 29$ DNA and that this is essential for transfection, seems remote since the circular protein-DNA complex of phage $\phi 29$ can be disrupted by treatment with sodium dodecyl sulphate³. Such DNA is active in transfection (F. K. and J. I., unpublished). Another possible explanation for our results would be that the DNA-protein complex is resistant to attack by nucleases present in competent cells. Although this possibility cannot be ruled out, it also seems unlikely since protease-treated $\phi 29$ DNA is able to rescue most of the genetic markers of $\phi 29$ mutants (F. K. and J. I., unpublished).

Instead, in analogy with the *coli* phage M13 system^{15,16}, it seems more likely that the $\phi 29$ *K* gene specifies a 'pilot' protein essential for $\phi 29$ DNA replication. The very same protein or a fragment thereof could be incorporated into the $\phi 29$ phage particle during phage maturation as an integral protein which is required for $\phi 29$ DNA transfection. Note that Ivarie and Pène¹⁴ found that after infection parental $\phi 29$ DNA is associated with host cell membrane. They described two mutants, one temperature-sensitive and the other suppressor-sensitive; both are unable to form DNA-membrane complexes and are defective in DNA replication¹⁷. Although the nature of the *K* gene protein and its function(s) are not known, further studies of this protein may clarify the role of DNA-associated proteins in DNA replication.

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Involvement of DNA in resistance of potatoes to invasion by *Phytophthora infestans*

THE primary event that determines whether or not a plant becomes diseased is a mutual recognition which brings the plant into contact with the pathogen. Flor¹ developed the gene-for-gene theory from his studies of the relationship between flax and rust. This theory implies that the genes in the plant that condition the reaction can be identified only by their interaction with specific races of the pathogen, and the genes in the pathogen that condition pathogenicity can be identified only by their interaction with a specific cultivar of the plant. Allen² reported that nucleic acid or protein was involved in the resistance of the plant to the pathogen. Ledoux *et al.*³ found that labelled DNA from bacteria could be incorporated into the nuclei of the plant cells. Thus DNA may have a role in the recognition of the plant by the pathogen. We have obtained support for this notion. We painted potato midribs and tubers with DNA fractions⁴ from different potato cultivars and species of Solanaceae, and found that their susceptibility to *Phytophthora infestans* was affected.

When petiole midribs of potato cultivar Norin No. 1 (gene *r*, for susceptibility) were inoculated with zoospores of *P. infestans* (race 0), only lesions were observed, but when the DNA fraction of the resistant interspecific hybrid 96-56 (gene *R₁*, for resistance) was applied to midribs of Norin No. 1, both lesions and hypersensitive flecks were found, and the cells invaded by the pathogen soon died (Fig. 1). Hypersensitive flecks are signs of premature dying off (necrosis) of the infected tissue as well as inactivation and localisation of the infectious agent, and are associated with the attack of a resistant plant by



Fig. 1 Lesions and hypersensitive flecks of late blight on potato tubers of Norin No. 1 painted with the DNA fraction of the hybrid. The DNA fraction was prepared by Thomas' method⁴. The concentration of DNA in the water solution was 455 $\mu\text{g ml}^{-1}$. Painting was done with a sterilised brush. *P. infestans* was inoculated 1 h after painting. ●—●, Lesions (control); ○—○, flecks (painted tuber); ○—○, lesions (painted tuber).

incompatible races of *P. infestans*; lesions result from the invasion of a plant by compatible races of the pathogen.

Results were similar with tubers of hybrid 96-56 in contact with the DNA fraction of Norin No. 1, but the control (Norin No. 1, not painted with the DNA fraction of hybrid 96-56) had only lesions. On the tubers of hybrid 96-56 treated with the DNA fraction from Norin No. 1, both mycelia and conidia developed, similar to those observed in the control. The conidia on the hybrid tuber were $25.9 \times 17.7 \mu\text{m}$, somewhat smaller than those of the control ($27.7 \times 18.1 \mu\text{m}$) (figures are the average of 50 conidia) (Fig. 2).

When petiole midribs of potato hybrid 96-56 were treated with the DNA fraction of tomato (cultivar Fukuju No. 2) both lesions and hypersensitive flecks were observed

Fig. 2 Mycelial growth of *P. infestans* on hybrid tuber painted with the DNA fraction of Norin No. 1. The concentration of DNA in the water solution was 457 $\mu\text{g ml}^{-1}$. *P. infestans* was inoculated 1 h after painting.

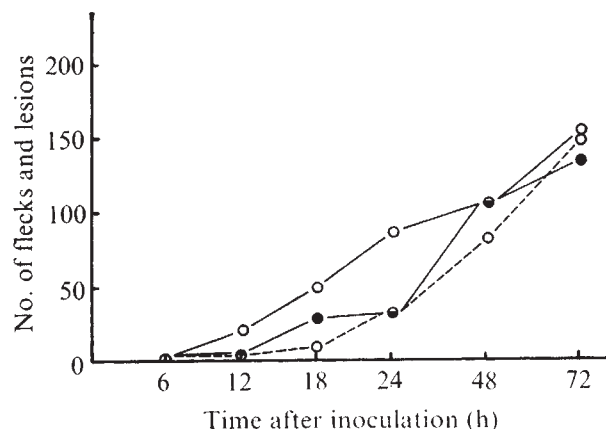


Table 1 Lesions and hypersensitive flecks of late blight on potato interspecific hybrid after treating with DNA fraction from tomato

	No. of flecks and lesions observed	No. of hypersensitive flecks	%	No. of lesions	%
DNA from tomato* on hybrid†	396‡	221	35.8	175	44.2
Hybrid (control)	229	229	100.0	0	0.0

*The concentration of DNA in the water solution was 433 µg ml⁻¹.

†Interspecific hybrid 96-56 (R₁ gene).

‡72 h after inoculation of *Phytophthora infestans* (race 0).

(Table 1). Similar lesions and flecks developed when the petiole of Norin No. 1 was treated with the DNA fraction of jimson weed (*Datura abla* Nees) and tobacco (*Nicotiana tabacum* L. cultivar xanthi nc), but in the control (Norin No. 1 without painting the DNA of jimson weed and tobacco) only lesions were found (Table 2).

Electrophoresis revealed an increase in the amount of protein in the leaves of the susceptible cultivar after treatment with the DNA fraction from resistant hybrid, before it was inoculated with pathogen. A new protein band, coinciding in R_F with a protein in the resistant hybrid, appeared 6 h after inoculation of an incompatible race of *P. infestans*. Potato leaves painted with the DNA fraction of different cultivars had more peroxidase and phenylalanine ammonia lyase activity than the control (without painting DNA).

Table 2 Lesions and hypersensitive flecks of late blight on potato painted with the DNA fraction from jimson weed and tobacco

	No. of flecks and lesions observed	No. of hypersensitive flecks	%	No. of lesions	%
DNA from jimson weed* on Norin No. 1	896*	309	34.5	587	65.5
Norin No. 1 (control)	781	0	0.0	781	100.0
DNA from Tobacco† on Norin No. 1	1,093	237	21.7	865	78.3
Norin No. 1 (control)	699	0	0.0	699	100.0

*The concentration of DNA in the water solution was 135 µg ml⁻¹.

†The concentration of DNA in the water solution was 300 µg ml⁻¹.

‡72 h after inoculation of *Phytophthora infestans* (race 0).

A large quantity of rishitin has been reported on the tubers of susceptible cultivars treated with the DNA fraction from the resistant hybrid⁶. Rishitin^{8,9} is one of several phytoalexins, which were believed to be important for the hypersensitivity⁷. Rishitinol⁹, lubimin¹⁰ and phytuberin¹¹ were also identified as phytoalexins in potatoes infected with *P. infestans*. Király *et al.*¹², however, showed that this hypersensitive necrosis was a consequence, not the cause of resistance of potato, beans and wheat to various fungi. We have described¹³⁻¹⁶, however, the role of DNA fractions of potatoes in the creation of hypersensitivity to invasion by *P. infestans*. These and our results support the idea that DNA is directly involved in the specificity of fungal pathogen for the plants.

Details of this work will be published elsewhere. We

thank Dr Y. Miyazaki for seeds of jimson weed and tobacco.

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New class of enzymes acting on damaged DNA

ONE of the major pathways of DNA repair consists of the removal and replacement of damaged nucleotides in non-replicating DNA. In the classical excision repair model^{1,2}, the first enzymatic step in this process is the introduction of a single-strand break in the DNA adjacent to a defective nucleotide residue. Endonucleases that specifically attack DNA containing pyrimidine dimers² or apurinic sites³⁻⁵ have subsequently been isolated from many types of cells, and enzymes of these two types have been shown to be active in DNA repair in *Escherichia coli* by the isolation of repair-deficient mutant strains with defective endonucleases^{6,7}.

An enzyme that specifically attacks DNA containing deaminated deoxycytidine monophosphate residues has also been detected in *E. coli*⁸. This enzyme is not, however, a regular nuclease, because it catalyses the hydrolysis of the N-glycosidic bonds of the damaged nucleotide residues, but it does not cleave any phosphodiester bonds. The reaction products after the action of this N-glycosidase on deaminated DNA are consequently free uracil and partly depyrimidinated DNA of unaltered chain length. Similar types of enzyme activities that release free 3-methyladenine (3-MeAde) and 6-methoxyguanine from alkylated DNA were discovered by Kirtikar and Goldthwait in a purified but non-homogeneous preparation of *E. coli* endonuclease II, and were ascribed to the endonuclease⁹. As the different DNA N-glycosidase activities may act together with endonuclease II in DNA repair, it seemed of interest to define the relationship of these enzyme activities to each other. In the present work, it is shown that the *E. coli* Ura-DNA glycosidase, 3-MeAde-DNA glycosidase, and endonuclease II activities are attributable to three different enzymes.

The three enzyme activities studied showed similar fractionation properties on streptomycin treatment, ammonium

sulphate fractionation, and gel filtration on Sephadex G-100 of an *E. coli* cell extract. On incubation at 45 °C of an enzyme fraction purified by these steps, however, the three activities were inactivated at different rates (Fig. 1). Endo-

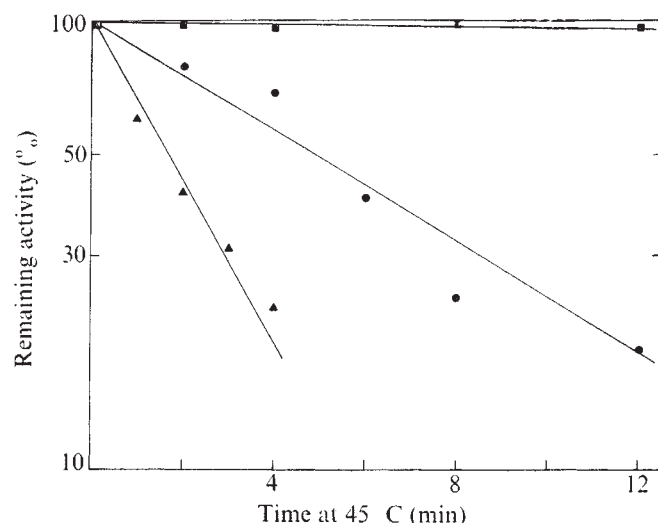


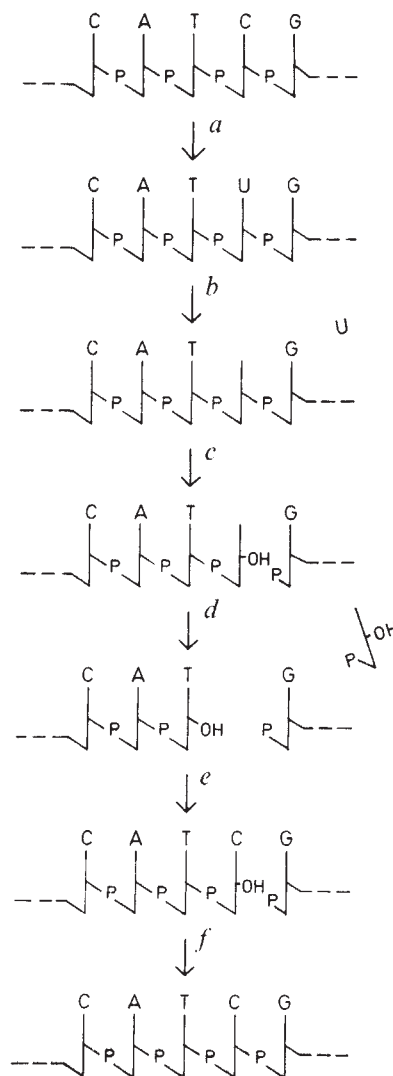
Fig. 1 Rates of heat inactivation at 45 °C of *E. coli* Ura-DNA glycosidase (■), 3-MeAde-DNA glycosidase (●) and endonuclease II (▲) activities. Activities were copurified from a crude cell extract of *E. coli* 1100 (endo I⁻) by streptomycin treatment, ammonium sulphate fractionation and gel filtration on Sephadex G-100 (ref. 8). Active protein fraction was dialysed against 0.2 M NaCl-0.05 M potassium phosphate (pH 7.4) and 0.001 M dithiothreitol, and aliquots were heated at 45 °C for indicated times before assay. Ura-DNA glycosidase was assayed by measuring the release of free uracil from partly deaminated DNA, initially radioactively labelled in the cytosine residues⁸. One enzyme unit releases 1 nmol uracil from DNA in standard reaction conditions. Endonuclease II was assayed by a modification of the method of Verly *et al.*^{4,16}. One enzyme unit releases 1 nmol ³²P in acid-soluble form from the radioactive, partly depurinated DNA substrate in standard reaction conditions. 3-MeAde-DNA glycosidase was assayed by measuring the release of free 3-MeAde from alkylated DNA (ref. 9), as follows. *B. subtilis* DNA, ¹⁴C-labelled in purine residues, was prepared as described previously¹⁷. DNA (0.3 mg ml⁻¹, 4 × 10⁷ c.p.m. mg⁻¹) was incubated with 0.25 M methyl methanesulphonate in 0.1 M Tris-HCl and 10⁻³ M trisodium citrate (pH 8.0) at 37 °C for 30 min. One-tenth volume 5 M NaCl and two volumes ethanol (chilled to -20 °C) were added at 0 °C, and the DNA fibres collected, washed in 80% ethanol, dissolved in 1 M NaCl, 0.01 M Tris-HCl and 0.001 M EDTA (pH 7.8), and dialysed for 16 h against the same buffer, followed by 3 h against buffer without NaCl. Alkylated DNA was stored in several small aliquots at -70 °C. This DNA contained 0.9% of the total radioactivity as 3-MeAde, as determined by paper chromatographic analysis of an acid hydrolysate in methanol-concentrated HCl-H₂O (7:2:1, ref. 18). 3-MeAde was quantitatively released from DNA by incubation at 100 °C for 10 min at neutral pH (ref. 18). In enzyme assays, the reaction mixture (25 µl) contained 0.07 M HEPES-KOH (pH 7.8), 10⁻³ M dithiothreitol, 2 µg alkylated ¹⁴C-DNA and an amount of enzyme sufficient to cause 10-50% release of 3-MeAde. After 20 min incubation at 37 °C, mixtures were chilled to 0 °C and individually supplemented with 5 µl 0.5% 3-MeAde (Cyclo Chemicals), 3 µl 5M NaCl, 10 µl 0.2% heat-denatured calf thymus DNA and 90 µl ethanol (chilled to -20 °C). After 1 h at -10 °C, samples were centrifuged at 2 °C, and 100 µl supernatant were recovered. This material, which contained <3% initial radioactivity, was applied to a Whatman 3MM paper at room temperature (21 °C) and analysed by descending paper chromatography for 17 h in a system containing a saturated ammonium sulphate solution, 0.1 M phosphate (pH 7.2) and isopropanol (79:19:2, ref. 19). After drying, 3-MeAde spot was localised by its ultraviolet absorbance, cut into pieces and transferred to a scintillation vial, and eluted with 5 ml H₂O for at least 1 h. Fifteen millilitres Aquasol (New England Nuclear) were then added, and the radioactivity was determined in a liquid scintillation spectrometer at a counting efficiency of 80%. One enzyme unit releases 1 nmol 3-MeAde in conditions described. In the absence of enzyme, 3% total 3-MeAde in the DNA was released by hydrolysis¹⁸ during incubation. The identity of the enzymatically released material with authentic 3-MeAde was confirmed in three additional chromatography systems.

nuclease II activity was most heat-labile, with 50% of the activity remaining after about 100 s. 3-MeAde-DNA glycosidase activity was inactivated at a rate three times slower and had 50% residual activity after 5 min. Ura-DNA glycosidase activity remained practically unchanged after 12 min at 45 °C, but was inactivated at 55 °C with 50% inactivation occurring after 3 min.

Direct evidence for the notion that Ura-DNA glycosidase activity depended on a separate enzyme was obtained by the physical separation of this activity from the other enzyme activities investigated by hydroxyapatite chromatography. When the Sephadex-purified enzyme fraction was applied to a hydroxyapatite column in a buffer containing 0.01 M phosphate, Ura-DNA glycosidase activity did not adsorb and was recovered in the effluent. In contrast, more than 90% of the 3-MeAde-DNA glycosidase and endonuclease II activities adsorbed to the column but were eluted with 0.3 M phosphate buffer (Table 1).

It has been shown that *E. coli* endonuclease II and exonuclease III are the same enzyme, which has been assayed by different methods⁷. In agreement with this notion, a preparation of exonuclease III, made according to Jovin *et al.*¹⁰, contained a high level of endonuclease II activity.

Fig. 2 Model for repair of partly deaminated or alkylated DNA. Complementary strand in DNA double helix not shown. *a*, Cytosine deamination (heat or bisulphite); *b*, release of free uracil (N-glycosidase); *c*, incision (endonuclease II); *d*, excision (exonuclease III); *e*, repair replication (a DNA polymerase); *f*, joining (DNA ligase).



In relation to the endonuclease activity, this enzyme preparation contained four fold less 3-MeAde-DNA glycosidase and 100-fold less Ura-DNA glycosidase activities (Table 1) than the much cruder Sephadex-purified enzyme fraction used here. This indicates that 3-MeAde-DNA glycosidase activity is partly removed and Ura-DNA glycosidase largely removed during the preparation of highly purified endonuclease II/exonuclease III by the above procedure.

In view of their specificities for damaged DNA^{8,9}, it seems likely that the DNA N-glycosidases are active in DNA repair. A hypothetical model for such repair is shown in Fig. 2. It is proposed that for some types of DNA lesions (excluding pyrimidine dimers), the specific recognition and elimination of the damaged base is due to an N-glycosidase. The apurinic (or apyrimidinic) site is then repaired by the action of endonuclease II/exonuclease III, a DNA polymerase and DNA ligase^{8,15}. One of the advantages of this

Table 1 Relative amounts of Ura-DNA glycosidase, 3-MeAde-DNA glycosidase, and endonuclease II in different *E. coli* enzyme preparations

Enzyme fraction	Ura-DNA glycosidase (units mg ⁻¹)	3-MeAde-DNA glycosidase (units mg ⁻¹)	Endonuclease II (units mg ⁻¹)
<i>E. coli</i> 1100, Sephadex G-100 fraction	1,050	7.0	230
Hydroxyapatite fraction A	4,100	1.5	< 5
Hydroxyapatite fraction B	50	8.0	210
<i>E. coli</i> AB1157 (<i>xthA</i> ⁺), Sephadex G-100 fraction	900	6.0	260
<i>E. coli</i> NH5016 (<i>xthA</i> ⁻), Sephadex G-100 fraction	1,100	6.5	25
<i>E. coli</i> exonuclease III (ref. 10)	300	50	7,000

E. coli Sephadex G-100 fractions were made as described⁸ (see also Fig. 1). For hydroxyapatite chromatography, an ammonium sulphate-concentrated Sephadex fraction (23 mg protein in 6 ml) was dialysed against 0.2 M KCl, 0.01 M potassium phosphate and 10⁻³ M dithiothreitol (pH 7.4), and applied to a column (1.3 × 11 cm) of hydroxyapatite¹⁰ in the same buffer. 20% of the protein did not adsorb to the column (fraction A). The adsorbed protein was then eluted with 0.3 M potassium phosphate (pH 7.4) and 10⁻³ M dithiothreitol (fraction B).

Several methyl methane sulphonate-sensitive *E. coli* mutants, defective in endonuclease II, have been isolated (ref. 7 and S. Ljungquist, T. Lindahl, and P. Howard-Flanders, unpublished). An enzyme preparation from one mutant of that type, *E. coli* NH5016, was investigated here together with a preparation from the parent strain, *E. coli* AB1157. As expected, the mutant had a tenfold decreased amount of endonuclease II activity. In contrast, the same mutant extract contained normal levels of both DNA N-glycosidase activities (Table 1). The above data strongly suggest that *E. coli* 3-MeAde-DNA glycosidase and endonuclease II are two different enzymes, and it is clear that neither of them is identical with the Ura-DNA glycosidase.

It has been shown with both *E. coli* endonuclease II and the similar mammalian endonuclease acting at apurinic sites that alkylated DNA is not a substrate, unless the DNA is first incubated to enable release of alkylated purines by hydrolysis^{4,11}. Goldthwait *et al.*¹² have claimed that the enzyme also acts directly on alkylated DNA. The finding of 3-MeAde-DNA glycosidase activity in partly purified *E. coli* endonuclease II preparations seems to explain this discrepancy, as the concerted effect of these two enzymes on alkylated DNA would be the introduction of single-strand breaks. Similar complications have arisen in the interpretation of data on enzymes degrading uracil-containing DNA. Carrier and Setlow¹³ showed that an apparent endonuclease activity that acted specifically on uracil-containing DNA was present in *Micrococcus luteus* extracts, but their experimental design did not separate between enzymes that introduce chain breaks and enzymes that introduce alkali-labile sites. Tomita and Takahashi¹⁴ reported that *Bacillus subtilis* contains a DNase specific for uracil-containing DNA, which released deoxyuridine as a major reaction product and was inhibited after infection with phage PBS1. The chromatographic method used to identify deoxyuridine, however, does not separate this substance from free uracil. Therefore, it seems unclear at present if conventional DNases exist that specifically attack uracil-containing DNA. Note that the Ura-DNA glycosidase seems to be a widely distributed enzyme that has so far been found in extracts from *E. coli*, *M. luteus*, *B. subtilis* and mammalian cells (T.L., unpublished).

scheme over the traditional excision repair model is that DNA ligase cannot act until the damaged nucleotide residue has been removed and replaced. A more definite assessment of the physiological functions of the DNA N-glycosidases, however, will have to await the isolation of mutant cell strains with defective enzymes of this class.

I thank Dr Bill Sugden for *E. coli* exonuclease III, and Ms Barbro Nyberg for assistance. This work was supported by the Swedish Natural Science Research Council, the Swedish Cancer Society and the Karolinska Institute.

Note added in proof: The rapid selective degradation of uracil-containing DNA by *B. subtilis* crude cell extracts is due to a Ura-DNA glycosidase. Extracts from phage PBS 2-infected bacteria contain a factor that completely inhibits the enzyme²¹.

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reviews

Ancient astronomical masterpiece

PTOLEMY'S (flourished AD 127–148) *Almagest* is a substantial and difficult work. Because it was the culmination of centuries of Greek astronomy, with its Babylonian background, and was the principal influence on astronomical theory up to the time of Copernicus and beyond, it has received the attention of every serious historian of astronomy. But, as Professor Pedersen says in his first chapter*, "it has been talked about by many, but studied seriously only by the few." His aim is "to help students of the history of astronomy to understand and appreciate Ptolemy's great and classical work."

Since the mathematical and astronomical ideas of the *Almagest* are not very abstruse, the difficulties lie in the mode of presentation. Some of these difficulties were pointed out in the twelfth century by Jābir b. Aflāh in his *Correction of the Almagest*. Among his many complaints, he said that the principles were obscured by numerical computations, that Ptolemy's exposition was sometimes too compressed, and that the meaning was often unclear because of the chain of translators between Ptolemy and himself. These defects Professor Pedersen has completely removed. He has elucidated the ideas behind Ptolemy's demonstrations, keeping them separate from numerical manipulations; difficult passages are examined in the light of recent scholarship; and, since he has worked from Heiberg's edition of the original Greek text, neither Professor Pedersen nor his readers have to suffer from the inaccuracies and obfuscations of translators.

Much of the clarity of this book comes from the author's ruthless use of modern notation. Not only are brackets, subscripts, square-root signs, and so on, used to describe the operations Ptolemy describes verbally, but some of his fundamental ideas are expressed in terms of functions, and some of his trigonometry is represented by the modern equivalent. Thus plane triangles are often solved in the modern way—that is, without dropping perpendiculars or circumscribing circles—and the functions *tan* and *cot*

are freely used where Ptolemy made do with chords. There is usually sufficient indication of what has been done. The result is that the reader knows that Ptolemy's exact mode of thinking has not been reported, but can see his line of reasoning without being distracted by the prolixities of archaic mathematical technique. It might be thought that modernisation is sometimes taken too far. On page 164, for instance, the mean longitude of the Moon is found by integrating its differential coefficient. While the reader may be assumed to realise that Ptolemy did not use the calculus, the modernity of the expression is startling and not really necessary. Elsewhere the anachronism is more than justified by the gain in clarity. For example, Ptolemy's calculation of lunar longitude from his general table is not easy to understand, but is fully explained in the *Survey* (pages 195–9) with the help of modern symbolism, particular attention being paid to Ptolemy's interpolative method of approximation. There are one or two mistakes in interpretation—I think, for instance, that the proof of the equivalence of the simple epicyclic and excentric models (pages 137–8) is mis-stated—

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Ptolemy taking the altitude of a heavenly body with a quadrant
(From G. Reisch, *Margarita Philosophica*, Basle, 1508)

but such lapses are rare.

In some cases, Professor Pedersen goes beyond what is written in the *Almagest*, but only to make the meaning clear. Several graphs of relevant functions are included to make the astronomical model under consideration easier to understand. Further aids for the reader are to be found in Appendix B, in which all the numerical parameters calculated in the *Almagest* are collected, and in the lists of observations. Besides a general inventory of dated observations (Appendix A), the observational data for particular astronomical models are tabulated in the appropriate place in the text.

The purely historical content of the *Survey*, although not so compendious as the expository, provides a useful introduction to Alexandrian astronomy and physics. There is a short chapter on Ptolemy's other works; and his reliance, in the *Almagest* and elsewhere, on Babylonian and Hipparchan observations and parameters is discussed as they occur. The book is introduced by a chapter on the transmission of the *Almagest* up to modern times. It is completed by a large and useful bibliography.

R. P. Lorch

* *A Survey of the Almagest*. (Acta Historica Scientiarum Naturalium et Medicinalium, Volume 30.) By Olaf Pedersen. Pp. 454. (Odense University: Odense, Denmark, 1974.) Dn. kr. 200.00

Solar radiation

Solar Gamma-, X-, and EUV Radiation. (International Astronomical Union Symposium No. 68, Buenos Aires 1974). Organised by the IAU in cooperation with COSPAR.) Edited by Sharad E. Kane. Pp. xii+439. (Reidel: Dordrecht and Boston, Massachusetts, 1975.) Dfl. 145; \$58.00.

DURING the past decade the rapid advance in instrumental techniques and the extensive use of orbiting observatories has provided a wealth of data on γ -, X-ray and EUV radiation from the Sun. The International Astronomical Union Symposium in June 1974 provided an opportunity for specialists in the field to review the observational results and to describe current theories of solar emission mechanisms and related phenomena. Highlights of the proceedings included reports of preliminary data from Skylab on the newly discovered coronal holes and bright points.

The book is split into three sections covering general solar activity, active regions and solar flares. Each section contains detailed review articles written

by leading authorities on the topics concerned, and summaries of contributed papers announcing the latest results. These include data from ground based telescopes, sounding rockets, balloons and satellites operated by American, European and Russian groups. In addition, the book contains papers on energetic particles and microwave radiation, forming a more complete picture of the solar phenomena in which they play a role. Thirty-nine papers are given in all.

The coverage of the rather broad subject suggested by the title is comprehensive, but inevitably in a collection of articles written independently by a large number of authors some duplication takes place and it is often difficult to refer to specific items of interest. The latter problem detracts a little from the value of the book as a reference volume for its intended audience of research workers and students in solar physics and solar terrestrial relations. Nevertheless, these workers will find it an important and necessary addition to the library of their respective research institutes, if a little expensive for their own bookshelves.

With the bulk of the Skylab data analysis yet to be completed and with the continued high level of activity in observational and theoretical solar studies it seems certain that this account of solar radiation will quickly be superseded. **C. G. Rapley**

but Lamarck comes in for much discussion.

I found some of the biochemical analogies in the book to be colourful, but perhaps not appropriate. For example, on page 268 the development of an egg into a vertebrate is likened to the proteolytic and lipolytic ripening of a cheese: "can develop as it were into a Cheddar, a Camembert, a Brie, etc., as well as into a Stilton".

Such an egg would become pidan rather than a duckling. The 'ripening' of cheese is not directed by the DNA inherently present, as in an egg. Ripening comes about by the degradative action of bacteria, as Waddington points out, by autolysis, or more usually by the growth of moulds. The "glory of ripeness" that he attributes to a Stilton cheese is the odour, taste and other properties of esters, fatty acids, and protein hydrolytic products that result from the decomposition of butterfat, lactose, casein and lactalbumin; a process that leads to putrefaction. What a contrast to the development of an egg—the fantastic spectacle of a DNA molecule at work to make another DNA molecule.

See also the description of protein structure on page 190, where the author says that all but "a small fraction of the amino acid chain" can be regarded as "packing or scaffolding and (has) not much more to do with the activity of the protein than had the scaffolding which allowed (the artist) to lie on his back... to paint the ceiling of the Sistine Chapel to do with the resulting artwork." This is probably an over-simplification of protein structure. For example, the enzyme, thermolysin, has a reactive site that is concerned with proteolysis, but the rest of the amino acid chain folds into a three-dimensional structure that permits only hydrophobic substrates to reach the reactive site.

Professor Waddington's discussions range across a wide scope of subjects, among them the evolution of developmental systems, adaptations, altruism, and language; genetic assimilation; canalising selection; the selection of environment *Drosophila* mutants; sexual isolation; archetypes in evolution; a catastrophe theory of evolution; and some interesting musings on "The human animal" and "The human evolutionary system"; and "Does evolution depend on random search?" His analysis of the "midwife toad incident"—a review of Koestler's book—is most entertaining.

The reader will find that Professor Waddington's book contains many fascinating excursions into evolutionary philosophy and into descriptions of the adaptations and special properties that make living organisms so enthralling to all of us. **T. H. Jukes**

MTP announce the publication of

The Biology of Cancer

A new approach
by Professor P. R. J. Burch

This book is a learned and closely-argued challenge to many current assumptions about the nature of malignant disease and the mechanisms of carcinogenesis. In particular it questions the widely-held presumption that smoking causes various cancers including lung cancer.

The book expounds and amplifies a theory of cancer and carcinogenesis that was first published in outline in *Nature* in 1970. A wealth of supporting evidence is cited from fields as diverse as molecular biology and clinical medicine.

This theory of cancer is the most detailed and comprehensive yet proposed: it also makes precise and testable predictions as well as suggesting some new approaches to the therapy of cancer.

January 1976
Price: £11.50

448 pages

MTP Press Ltd.,
St Leonard's House,
Lancaster, LA1 1PE

Evolutionary thoughts

The Evolution of an Evolutionist. By C. H. Waddington. Pp. xii+328. (Edinburgh University Press: Edinburgh, May 1975.) £6.00.

PROFESSOR Waddington has reprinted twelve of his journal articles (five of them from *Nature*), and twelve excerpts by him from textbooks and symposia volumes. To these he has added two exchanges of correspondence (with T. Dobzhansky and P. M. Sheppard) and four book reviews. Two brief essays, not published elsewhere, are also included. The time span of the material reprinted covers from 1941 to 1974. Some of the subject matter is concerned with *Drosophila* genetics, and there is also a great deal of discussion of the differences between various trends of evolutionary thought.

I have a feeling that the question of the inheritance of acquired characters, so much a target of discussion and refutation by evolutionists, was dealt a mortal blow by the discovery of the molecular mechanism of protein synthesis. This point is not mentioned,

UNACUSTOMED as I am to watching television, it was with some diffidence that I agreed to review *The Human Conspiracy*, a programme that is part of a series intended sporadically to bring the latest scientific developments to your fireside. My experience while watching reminded me of those of a wealthy friend who always insisted on travelling first class. He had been brought up to believe that the most terrible goings on occurred in the second class and on the only occasion when he ventured to pass into that part of a ship, his worst expectations were confirmed. He wandered down a gangway and seeing an open door, entered a cabin just in time to cut down a passenger who had attempted to hang himself, doubtless driven to desperation by conditions in steerage. Whether this programme is typical of the BBC or whether it represents an isolated attempt at self destruction, it is not for me to say.

The programme consisted of a series of fragmentary episodes loosely organised around the theme of how men and women get to be the way they are. Viewers were shown psychologists in Belgium posing an impossible problem to a prisoner to assess his reactions, although just how this would help to determine whether he should be released was not made clear. A sequence showing a schizophrenic and her identical twin in Bergen led up to the misleading statement that "for schizophrenia it's two-to-one environment versus genes"—Ladbroke's please note. Malnourished children in Mexico were presented to demonstrate that starvation can lead to irreversible losses in intelligence. Liberian children have difficulty with mathematics, although their mothers are not surprisingly better at judging the amount of rice in a bowl than American college students. X-ray pictures of a foetus in the womb showed that babies are sensitive to their environment long before they are born. This sequence was duly followed by a picture of a baby being born but we were not told what this illustrated. We were then treated to innumerable shots of babies breast feeding, crying, following objects with their eyes, and, of more interest, howling at the sight of three simultaneous images of their mothers.

The second half concentrated on social interactions. Ron Moody donned a long nose to demonstrate that people tend to shun actors wearing false noses. Boys are more aggressive than girls, probably because they have more testosterone: so bring on a male chimpanzee displaying; and to illustrate that self-sacrifice is not merely a human virtue, why not have some soldier ants sacrificing themselves in defence of their queen? Robert Trivers discussed

the concept of reciprocal altruism which purports to explain why it is that men can be kind to people outside their immediate family: "if you scratch my back I'll scratch yours and therefore we shall both live to propagate our genes". Shots of poor whites in Pennsylvania and of Maori children illustrated the problem of being a member of a group whose identity is threatened, and the Trobriand islanders

Missing connections

The Human Conspiracy (BBC2, Saturday, December 6) set out to investigate new discoveries and theories about our personal and social behaviour. N. S. Sutherland here reviews the programme with some reservations. The still below shows presenter Caroline Medawar.



were held up as a society free from class hatred and loneliness, and in which man's status depends on how much he gives not how much he takes, although how anyone gets enough in the first place to go on giving was never explained. Finally, war scenes were used to depict the idea that loyalty to a group may result in savage cruelty to out-groups, and the programme ended with the thought that "More than brotherly love, it's courtesy that makes the world go round".

Miss Caroline Medawar was the comère and very school-marmish she was: she gave the impression of having learned her lines carefully by heart and it is not clear whether her stilted intonation arose through failure to understand them or because she wished to dissociate herself from their silliness: she should perhaps be given the benefit of the doubt. The male commentators were more fortunate since they were spared the embarrassment of actually having to appear. Much of the visual material had little to do with the intellectual content and on the principle of shooting whatever moves, shifts to new locations were frequently accompanied by shots of motor cars.

I appreciate the difficulty of devising

a scientific programme intended for a general audience. The most successful attempts, to my mind, have been programmes introduced and informed by a single scientist with sufficient charisma to project his own personality: one example is the late Professor Korner's excellent series on molecular biology. Even where the contents are rather scrappy, as in Bronowski's *Ascent of Man*, the glimpse of an individual mind at work does something to provide a sustaining connection. I suspect that the producers of *The Human Conspiracy* have underestimated the intelligence and staying power of a general audience: a programme that concentrated on a single theme and made explicit the conclusions reached and the research methods used would surely be more gripping than one that makes a series of disconnected points none of which are developed in depth.

Moreover, the practice of taking all the examples from ongoing research is misleading, and gives the viewer a spurious impression that he is being presented with new conclusions: we were given no hint that research on in-groups and out-groups has been under way for many years and I could not grasp what new findings were expected to emerge from much of the current research shown. By limiting the programme to such research, the producers lose the opportunity to choose the study that best proves a point, where that study, as is usually the case, has already been completed. Finally, there can be no excuse for perpetrating scientific howlers. It was argued, for example, that since homosexuals cannot transmit their own genes, they only survive because they are of use to their immediate kin. There is, however, no evidence whatsoever that homosexuality is an inherited condition, and the argument is therefore vacuous.

I realise that the programme was not intended for readers of *Nature* and the writer, Nigel Calder, once claimed to follow Faraday in thinking that the "generality of mankind cannot accompany us for one short hour unless the path is strewn with flowers". This patronising remark captures the spirit in which the programme was devised, but even if it were true, some effort might have been taken to provide garlands in a healthy state of bloom rather than dead or wilting blossoms. I personally would have preferred a few brambles to relieve the bland and cosy monotony, typified by a sentiment expressed by Professor Jerome Bruner: "Isn't it clever of Nature to arrange that play like most other things in life doesn't work unless there's some fun in it". Ugh. □

announcements

Awards

The Award Program in Cancer Immunology of the Cancer Research Institute of New York gave its inaugural prizes in October 1975 to: Dr Hans O. Sjogren, University of Lund, Sweden; Dr Robert J. Huebner, National Cancer Institute, Bethesda, Maryland; Dr Lloyd J. Old and Dr Edward A. Boyse, Sloan-Kettering Institute for Cancer Research, New York; Mr Edward J. Foley, Schering Corporation, Bloomfield, New Jersey; Peter A. Gorer (posthumous award); Dr Edmund Klein, Roswell Park Memorial Institute, Buffalo, New York; Dr George Klein, Karolinska Institutet, Stockholm, Sweden; Dr Richmond T. Prehn, University of Pennsylvania; Dr Gertrud Henle, Children's Hospital, Philadelphia, Pennsylvania; Dr Robert A. Good, Sloan-Kettering Institute for Cancer Research, New York; Mrs Helen C. Nauts, Cancer Research Institute, Inc., New York; Dr Donald L. Morton, University of California, Los Angeles; Dr Ludwik Gross, Veterans Administration Hospital, Bronx, New York; Dr Werner Henle, Children's Hospital, Philadelphia, Pennsylvania.

The British Pharmacological Society has awarded the Sandoz Prize in pharmacology to Dr J. Hughes of Aberdeen University for his pioneering work on encephalin.

The Dr H. P. Heineken Prize for 1976 has been awarded to Professor L. L. M. Van Deenen of the University of Utrecht for his research on the mechanism of action of phospholipases and on the structure of biomembranes.

Appointment

Professor Yosef Aloni has been appointed the first Duckwitz Professor at the Weizmann Institute. The chair, for cancer research, has been established by the Federal German government in honour of Georg F. Duckwitz.

Reports and publications Great Britain

Agricultural Research Institute of Northern Ireland. Forty-eighth Annual Report, 1974/1975. Pp. 49. (Hillsborough, Co. Down: The Agricultural Research Institute, 1975.) [411]
Social Consequences of the Energy Situation—Report of a Study Group. (British Association Publication 75/1). Pp. 15. (London: British Association for the Advancement of Science, 1975.) 50p. [611]
An Introduction to Liquid Scintillation Counting; and Solutes and Solvents for Liquid Scintillation Counting. Combined edition. By Dr. J. B. Birks. Pp. 41. (Colnbrook, Bucks: Koch-Light Laboratories, Ltd., 1975.) *Gratis*. [611]
Potato Marketing Board. Annual Report and

Accounts, 1975. Pp. 36. (London: Potato Marketing Board, 50 Hans Crescent, SW1, 1975.) [611]
Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 272, No. 915:

The Medical Research Council's Steroid Reference Collection has recently received a most generous gift from Professor T. Reichstein, Basel, of some four hundred samples from his extensive collection of steroids, which represents the results of forty years' research. Compounds in the androstane and pregnane series will be included in the next list of additions to the Steroid Reference Collection.

A large part of the gift consists of a unique range of derivatives of bile acids, of the types studied intensively for partial synthesis of adrenocortical steroids. These compounds range from the 'natural' bile acids of the C-24 (cholic acid) series, through nor-acids (C-23) and dinoracids (C-22), down to the etianic acids of the C-20 series. Within each series there are representatives of unsubstituted types, 3-, 11-, and 12-monosubstituted types, 3,7-, 3,11-, 3,12- and other disubstituted types, and finally 3,7,12-trisubstituted types.

Lists of these materials are available to workers who are interested and who can show a direct need for specific compounds; applications should be made to Professor W. Klyne or Dr D. N. Kirk at Westfield College, Hampstead, London NW3 7ST.

The amounts of material available in some cases are small, and many samples represent a considerable part of the world's supply of the compound. In these circumstances the Steroid Reference Collection must exercise restraint in the issue of such valuable materials.

The Foulkes Foundation invites applications for its 1976 fellowships. They are intended to finance science graduates taking a medical degree before doing medical research, or, similarly, medical graduates needing an additional science degree. Those interested should contact D. W. FitzSimons, Secretary, The Foulkes Foundation Fellowships, The CIBA Foundation, 41 Portland Place, London W1N 4BN.

A Discussion of the Physics and Chemistry of Biological Recognition. Organized by D. C. Phillips, and G. C. Radda. Pp. 1-198 + plates 1-3. UK £8.40; Overseas £8.65. Vol. 272, No. 916: Early Seed Development in the Triticeae. By M. D. Bennett, J. B. Smith and I. Barclay. Pp. 199-227 + plates 1-3. UK £1.60; Overseas £1.65. (London: The Royal Society, 1975.) [611]

A Bibliography of Geodynamics—oriented Publications by Scientists in the United Kingdom, 1970/1975. Pp. 38. (London: The Royal Society, 1975.) [611]
Wildlife Conservation and Lichens. By O. L. Gilbert. Pp. 16. (Exeter: The Devon Trust for Nature Conservation, Ltd., 1975.) [711]
Land Economy: An Education and a Career. By D. R. Denman. (A Lecture delivered at the 137th Annual Meeting of the British Association for the Advancement of Science, 1975.) Pp. 32. (Berkhamsted, Herts: Geographical Publications, Ltd., 1975.) 50p. [711]

Tobacco Consumption in Various Countries. Edited by P. N. Lee. Compiled by M. J. Wilson. Pp. 86. (Research Paper 6.) 4th edition. (London: Tobacco Research Council, Glen House, Stag Place, SW1, 1975.) [711]

Professional Engineers, Scientists and Technologists in the Engineering Industry. By Muriel Vennings. Pp. 150. (Research Report No. 4.) (Watford, Herts: Engineering Industry Training Board, 1975.) [711]

Research Supported by the Social Science Research Council, 1975. Pp. 433. (London: Social Science Research Council, 1 Temple Avenue, EC4, 1975.) £1.50. [1011]

The Peak District National Park: The Way Ahead. Pp. 12. (Bakewell, Derbyshire: Peak Park Planning Board, 1975.) [1111]

An Introduction to the Open University. Pp. 24. (Milton Keynes: The Open University, 1975.) [1111]

The Western European Energy Economy: Challenges and Opportunities. By Professor Peter R. Odell. (The Stamp Memorial Lecture delivered before the University of London on 13 November 1975.) Pp. 40. (London: University of London, The Athlone Press, 1975.) [1211]

Commonwealth Bureau of Nutrition. Protein-Calorie Malnutrition of Early Childhood: Two Decades of Malnutrition. A Bibliography compiled by E. F. Patrice Jelliffe. Pp. 118. (Farnham Royal, Slough: Commonwealth Agricultural Bureaux, 1975.) [1211]

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 272, No. 917: The Genetics of the Mimetic Butterfly *Hypolimnys bolina* (L.). By Sir Cyril Clarke and P. M. Sheppard. Pp. 229-265 + plates 1 and 2. UK £2.25; Overseas £2.35. Vol. 272, No. 918: A Discussion on the Results of the 1971 Royal Society—Percy Sladen Expedition to the New Hebrides. Organized by E. J. H. Corner and K. E. Lee. Pp. 267-486 + plates 1 and 2. UK £9.10; Overseas £9.35. (London: The Royal Society, 1975.) [1311]

Wildfowl 26. Edited by G. V. T. Matthews and M. A. Ogilvie. Pp. 176. (Slimbridge, Gloucestershire: The Wildfowl Trust, 1975.) £2.75; \$8. [2011]

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 272, No. 919: The Intrinsic Association and Commissural Connections of Area 17 of the Visual Cortex. By R. A. Finken. L. J. Garey and T. P. S. Powell. Pp. 487-536 + plates 1-21. (London: The Royal Society, 1975.) UK £4.75; Overseas £4.90. [2011]

British Antarctic Survey. Scientific Reports. No. 51: The Geology of the Duse Bay—Larsen Inlet Area, North-East Graham Land (With Particular Reference to the Trinity Peninsula Series). By Dr. N. Aitkenhead. Pp. 62 + 12 plates. £6 net. No. 83: A New Assemblage of Plant Fossils from Milorgfjella, Dronning Maud Land. By Dr. Edna P. Plumstead. Pp. 30 + 15 plates. £2.60 net. (London and Cambridge: The British Antarctic Survey, Natural Environment Research Council, 1975.) [2111]

Journal of Thermal Biology, Vol. 1, No. 1, October 1975. Edited by K. Bowler and J. E. Heath. Pp. 1-60. Published quarterly. Annual Subscription Rates 1975: \$35; Individuals whose institution takes out a library subscription may purchase a second or additional subscription for personal use at \$25. (Oxford and New York: Pergamon Press, 1975.) [2611]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 280, No. 1294: Metamorphic Processes at High Temperature and Low Pressure—The Petrogenesis of the Metasomatized and Assimilated Rocks of Carneal, Co. Antrim. By P. A. Sabine. With X-ray Studies by B. R. Young. Pp. 225-269. (London: The Royal Society, 1975.) UK £2.15; Overseas £2.25. [2711]

Chemical Industries Association Limited. Activities Report 1973/1975. Pp. 16. (London: Chemical Industries Association Limited, 1975.) [2711]

The Royal Society. Report of Council for the year ended 31 August 1975. Pp. 118. (London: The Royal Society, 1975.) [2811]

Science Research Council. Advanced Ground Transport—a Review of Research Possibilities. Pp. 73. (London: Science Research Council, 1975.) *gratis*. [2811]